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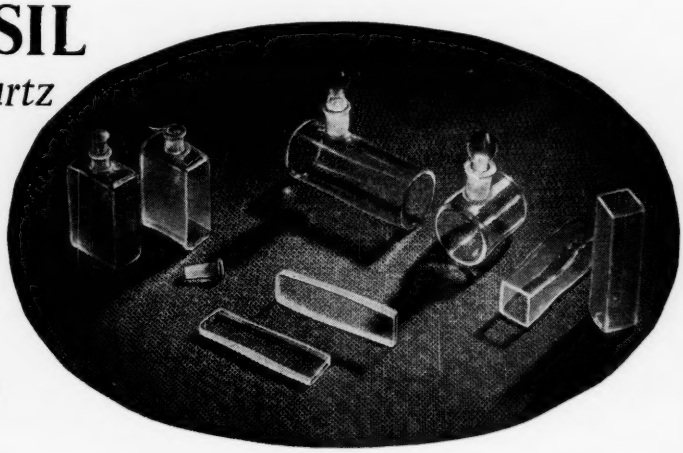

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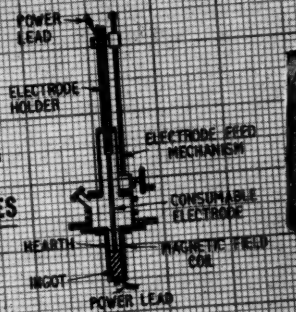

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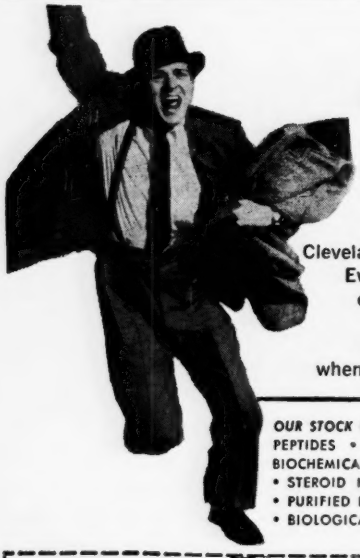

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NATURE

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SECONDARY AND HIGHER EDUCATION IN BRITAIN

A PAPER entitled "A Policy for Higher Education", published as a special supplement in *Socialist Commentary* of September over the signatures of Mr. Robin Morris as chairman, J. Vaizey and A. H. Halsey as rapporteurs, the co-signatories including Prof. W. J. M. Mackenzie and M. Stewart, M.P., is the outcome of three conferences of people from different but appropriate professions, and while representing a compromise between various lively points of view, it is intended as a basis for further discussion. It supports the idea of a Royal Commission on institutions of higher education in Britain, but emphasizes the importance of prior thinking if such a Commission is to be effective, and also urges that any such Commission should consider the whole field of higher education rather than concentrate on the universities themselves. The paper is not itself concerned directly with secondary education, but its signatories are agreed as to the need for considering carefully the interactions of secondary and higher education.

The primary objective of the paper appears to be to ensure equality of opportunity in education, and the writers recognize that this must be based on equality of opportunity in secondary education. The argument and implications of Michael Young's essay, "The Rise of the Meritocracy", are clearly in mind, and in the phrase 'equality of opportunity' the last word is generously interpreted. One of the major recommendations—the amalgamation of teacher training colleges and colleges of technology into colleges of liberal arts and technology enjoying the autonomy and relation to the community which characterizes the universities proper, and administered by a body analogous with the University Grants Committee but in touch with the work of the local authorities in this field—is intended to meet this need. Nevertheless, the paper appears to be somewhat doctrinaire and would have been more convincing had it been based on a thorough and impartial inquiry into some of those matters such as the size of classes, the effect of lengthening the period of compulsory schooling at one end rather than the other, the advantages or disadvantages of large schools as against co-education and of training teachers for three years consecutively, which, in the absence of precise knowledge, are liable to be the sport of political theory or prejudice.

On the expansion of higher education, a target of 300,000 full-time students by 1970 is recommended, and of these about one-third should be in technical and teacher training colleges. This would involve an increase of 6 or 7 per cent per annum in the number of teachers if present staffing ratios are to be maintained, and in this context it is recommended that improvement of the economic position and status of teachers in universities and institutions of higher education (outside Oxford and Cambridge) is essential. The report recommends less specialization at school, but while the signatories may well be right in suggesting that some sixth-form teachers would be more advantageously employed in a college or university, the proposal to shift educational effort from the sixth form to the university is more debatable, and should be based on facts—some of which have still to be determined—rather than theory. The economics of any such step are dubious and also its social consequences, quite apart from whether it is really consistent with the true functions of a university.

To some extent that is also true of the recommendation that expansion of the provincial universities of Britain should be accompanied by rising standards in staffing ratios, salary scales, buildings, halls of residence, etc., directed at bridging the gap between 'Redbrick' and 'Oxbridge'. Heavily increased expenditure on the provincial universities may well be overdue, but this is not necessarily the only, or even the best, way to enable them to attract a larger proportion of the first-class minds in each generation of school-leavers. The institution of a council for higher education to determine the allocation of funds for higher education is also recommended, but its position with regard to the University Grants Committee is somewhat ambiguous and no convincing evidence is presented that a new body is required rather than some re-arrangement or expansion of the functions of the latter. Although it is recommended that each university should be encouraged to act as a teaching and research centre for the college of liberal arts and technology of its region, the question of technology is very superficially considered; and in seeking to break away from the conception of a university as a place providing specialized training for professional and highly scientific occupations towards one providing general education of a high standard, the signatories of the report seem to become somewhat confused and involved in contradictory policies.

Much more fundamental thinking about the purpose and functions of a university is thus required to resolve the issues that are raised in this section of the paper. The conception of the university as a place of learning and research as well as of teaching seems to have been overlooked, and while the recommendations on awards and grants are sound, the emphasis on the claims or rights of the individual, particularly in relation to admissions, seems to be excessive and to lose sight of obligations and responsibilities.

A national university entrance examination, taken in March, from the results of which individual institutions would select their entrants without further test, is suggested. Most of the group wish the Government to use financial pressure to reduce the proportion of entrants to Oxford and Cambridge from private schools; but the minority thought this was more likely to increase than to diminish the predominance of Oxford and Cambridge, and recommends rather that these Universities should no longer

enjoy subsidies from public funds. The group recommends that all university students should pass through a diagnostic year of general education before being allocated to suitable courses. It does not discuss the practical implications of thereby extending university education to a fourth year, nor does the feasibility of this year of general education being given in the sixth forms of schools appear to have been considered. Again, it is taken for granted that wide adoption of the tutorial system in higher educational institutions and the consequent high staff–pupil ratio are justified. More experiment in teching methods would be welcomed, but the urgency of such an inquiry, as a preliminary or an essential part of university expansion, is nowhere emphasized ; and although it is recognized that heavy teaching duties involve some easing of the demands of research and administration, the complexity of the issues raised thereby is dismissed too lightly. It is admitted that fresh thought is required on the subject of halls of residence, but the thinking of the group here appears to have been guided too much by its political preconceptions, and the part of the library is nowhere mentioned.

Nevertheless, in the section of the paper dealing with residence, there is some realistic thinking, though even here the group has its inhibitions, and its assertions that residence in hall prolongs adolescence and that such students are subsidized at the expense of other students could be challenged. The final section, however, with its emphatic recommendation that a comprehensive further education service should be established, is altogether admirable. Such a service should envisage the amalgamation of the youth employment and youth services, and the group suggests that the county colleges, for which provision was made in the 1944 Act, would constitute the natural headquarters, offering membership to every youth not otherwise enrolled in an institution of full-time further education. There would also be close links with the colleges of liberal arts and technology and with the extra-mural departments of the universities.

It is perhaps for this recognition that any sound policy must treat higher education as a whole and that its development must equally be planned in relation to secondary education and indeed primary education that the paper is most important. The paragraph in the Queen's speech dealing with education indeed referred to the schools as well as to technical colleges and universities, and at least implied that the supply of teachers is a common problem. A specific reference to the reduction in the size of classes might suggest that this is a priority in the Government's policy ; but nothing was said in the debate on the Queen's speech to indicate that the independent investigations are already in train upon the results of which the priorities should be based. References to education in the House of Commons debate related mainly to the youth service and to the modernization of school buildings, and in replying on a debate which had been concerned mainly with the youth service, the Minister of Education, Sir David Eccles, conceded that in the last Parliament technical education had received the greatest attention. He said it is now time to give greater attention to the needs of the fifteen- to eighteen-year-olds, especially in leisure and in non-vocational education.

Sir David pointed out that this in itself brings us back to the primary schools and that we are concerned with the needs of some 7 million children and 300,000 teachers in all sorts of schools and colleges. The Government is fully determined, he said, to make a major advance in the youth services and the leisure activities of young people ; but he gave no indication of either the magnitude of the effort or how the priorities are to be assessed.

Although education received rather less attention in the debate on the Queen's address in the House of Lords, the Earl of Home in a reply for the Government was a little more forthcoming. He welcomed Lord Rea's reference to our need to equip the young to meet the intellectual, physical and moral challenge of the time and, while admitting that the expansion contemplated for the universities and the technical colleges would strain our resources, agreed that it nevertheless must be made. Education, however, must look beyond technology, and Lord Home agreed with Lord Hastings on the need for constructive and imaginative thinking if the right solution to our problem is to be found. The mere expenditure of £700 million, a figure expected to rise for England and Scotland to £1,000 million a year by the early 1960's, is improbable of itself to suffice. We are unlikely ever to have the resources to do what is desirable at any one time. The selection of priorities is unavoidable, and what matters is that the right priorities should be chosen.

"A Policy for Higher Education" challenges the critical thinking and inquiry which should precede the choice of priorities and which should indicate the right priorities. Its main emphasis is on higher education, but not on higher education without regard to the needs of secondary education or of further education in the more general sense. The need for balance is recognized, and the discussion which it seeks to provoke should elucidate some of these issues and may even stimulate the impartial inquiry that is needed to collect the facts on which decisions should be based. Not least, apart from re-examination of the inter-relations between school and university, the paper should stimulate some fresh thought about the extra-mural work of the universities and their regional responsibilities.

MAGNETOHYDRODYNAMICS

Cosmic E'ectrodynamics

By Dr. J. W. Dungey. (Cambridge Monographs on Mechanics and Applied Mathematics.) Pp. ix + 183. (Cambridge : At the University Press, 1958.) 32*s*. 6*d*. net.

A DECADE has elapsed since the publication of Alfvén's book, "Cosmical Electrodynamics"—a subject which was then beginning to attract the attention of the scientific world. Since then, the new science, now generally called 'magnetohydrodynamics', has grown in importance and interest and there exists

to-day a vast literature on the subject—a fact which must be particularly gratifying to its originator. The book under review, bearing almost the same title as Alfvén's book, gives some indication of the progress and of the consolidation which have been achieved in this field during the intervening decade; and while Alfvén inevitably confined himself for the most part to his own pioneer researches, Dungey has cast his net wider and, as was to be expected, his approach is more sophisticated.

The book effectively consists of two parts, although the author makes no such division. The first part deals with magnetomechanics proper, and some interesting causal general relationships are discussed at length. Certain pitfalls which abound in this subject are pointed out—notably the fallacies into which one may be drawn by an injudicious application of the orbital motions of an isolated charged particle in a magnetic field to the behaviour of an ionized gas in a magnetic field.

The second part of the book is concerned with applications to solar and terrestrial magnetism (mainly magnetic storms and aurorae), ionospheric electrodynamics and the author's own discharge theory of solar flares.

There can be no doubt that the author has given an admirable synthesis of the subject. The mutual interaction of the mechanical forces and the electromagnetic forces in a conducting fluid moving in a magnetic field is fully and clearly discussed, and attention is given to the important problem of the stability of twisted magnetic fields.

One of the more important problems in that field, the stability of plasma equilibrium configurations, which has figured prominently in recent years in connexion with the problem of controlled thermonuclear reactions, is here discussed by the author mainly from the point of view of astrophysical applications. Nevertheless, some attempt is made to discuss the problem more generally in the chapter on magnetodynamics under the section on small oscillations. Brief references are also made to hydromagnetic shock waves and hydromagnetic turbulence.

There is an admirable account of solar phenomena, including granulations, sunspots, surface magnetic fields, prominences, flares and *M*-regions. But the author seems to have been reluctant to supplement this by a more extensive account of current theories of sunspot magnetic fields; and the dynamo theory of the origin of the geomagnetic field is not treated as fully as one might have expected.

The chapter on magnetic storms and aurorae, one of the most successful in the book, contains a fairly complete account of one of the current theories of these phenomena. It is a pity that the author has not seen fit to make some reference to the theories developed by Alfvén and Singer, probably because Alfvén himself has given a full account of his theory in his own book. Both theories contain many fruitful ideas, and the recently discovered Van Allen trapped-particle radiation appears to have been uncannily prophesied in Singer's work.

There are few errors in this book; in the opinion of the reviewer it gives one of the most comprehensive and accurate accounts of magnetohydrodynamics yet published. It is admirably constructed, and though it is not an easy book to read, the reader will nevertheless be amply rewarded by the many interesting and stimulating ideas he will find in it; it should be read by everyone interested in the subject.

V. C. A. Ferraro

DEVELOPMENT OF NUCLEAR POWER

The Economics of Nuclear Power, including Administration and Law

Vol. 2. Edited by I. R. Maxwell, P. W. Mummery and Philip Sporn. (Progress in Nuclear Energy, Series 8.) Pp. vi+419+xii. (London and New York: Pergamon Press, 1959.) 105*s*. net.

Partnership for Progress: International Technical Co-operation

Being the *Annals of the American Academy of Political and Social Science* (May 1959).

THE fifth annual report of the United Kingdom Atomic Energy Authority referred particularly to the need to relate plans for the development of reactors to the possibilities of the export market and pointed out that, although much of the Authority's work on the larger systems will have application to small reactors, considerable advances will be required in nuclear technology before reactors of the range 20–100 MW. become competitive in normal circumstances. It is in this context that a number of the papers contributed to the second International Conference on the Peaceful Uses of Atomic Energy which have now been published are of some general interest. Some of those dealing with the economics of the generation of nuclear power, like those of J. A. Jukes on the economics of nuclear power, of J. C. Duckworth and C. Hywel Jones on economic aspects of the United Kingdom nuclear power programme, have in a few points been modified by statements in Parliament or elsewhere.

Three papers offer an important contribution to the discussions of power problems in the undeveloped countries. Two of these are concerned with the world demand for nuclear energy: in the first, K. M. Mayer reviews the economic setting for nuclear power and heat development, and in the second, Dr. H. J. Bhabha discusses the need for atomic energy in the under-developed countries. With N. B. Prasad he also surveys the contribution of atomic energy to a power programme in India. Besides these, the Atomic Energy Commission of Japan describes the long-term plan for the development of atomic power reactors in Japan authorized in 1957.

This plan emphasizes the large number of scientists and engineers required for technical research on and the manufacture of reactors, fuel elements and related equipment, and recognizes that there is a scarcity of such men throughout the world. With this difficulty Dr. Bhabha does not appear to be concerned, and to him the problem is one rather of economics and capital investment. It is probable, however, that the question of scientific and technical man-power, and the related one also recognized by the Atomic Energy Commission of Japan of a national industrial structure which permits efficient and easy assimilation of nuclear reactors and equipment, are far more important than finance. It is at least clear that the effective exchange of information and scientists and the establishment of close international co-operation, of which the Geneva Conference and the publication of its edited proceedings is an outstanding example, are essential factors in all such developments.

The development of nuclear power more especially in the under-developed or less advanced industrial countries is, however, only a particular example of international technical co-operation, and the May

1959 issue of the *Annals of the American Academy of Political and Social Science* devoted to that theme should be of even wider interest. It shows the perspective in which technical co-operation is seen by Americans whether from the Congress end or from within a particular mission. The characteristic approaches of the United Nations Expended Programme and of the United States Point Four Programme are next outlined and then the less well-known bilateral approach of the Sino-Soviet Bloc technical assistance.

The bulk of the Symposium, however, is concerned with problems of administration, and these are illustrated finally by descriptions of three programmes: the United Nations programme in Brazil; the United States operations mission in Iraq; and the Ford Foundation programme in Pakistan. The problem of finance is not ignored and Dr. L. K. Caldwell deals imaginatively with the role of the technical expert, as does Mr. R. W. Jones with the problem of personnel management in this field. Constructive suggestions are to be found in the papers by P. M. Glick on the choice of instruments for technical co-operation, by H. Reining on the Government contract as an administrative device, and by G. W. Lawson on technical co-operation for administrative improvement. By and large the papers cover from an American angle ground already traversed by Political and Economic Planning, and while they do not offer much in the way of new proposals they show the extent to which there is common ground in much progressive thought in this field, some directions in which further study and creative thought are needed and the magnitude of the effort which is already being made, to which in a special field some of those papers contributed at the Geneva Conference on the Peaceful Uses of Atomic Energy also testify.

R. Brightman

ROCHESTER CONFERENCE ON SEMICONDUCTORS

Advances in Semi-Conductor Science

Proceedings of the Third International Conference on Semi-Conductors, held at the University of Rochester, U.S.A., August 18–22, 1958. Edited by H. Brooks. (International Series of Monographs on Semiconductors.) Pp. ix+553. (London and New York: Pergamon Press, 1959.) 100*s*. net.

THE report of a conference can unfortunately be little more than a collection of papers which could have appeared several months earlier, distributed among a handful of journals, and can be devoid of any of the spontaneous interchange of ideas which a conference should promote. That of the Rochester conference on semiconductors does, however, include summaries of the discussions concluding each session, a review by J. Bardeen of the trends in semiconductor research emphasized by the conference, and five excellent descriptive summaries by H. Brooks, D. Polder, L. Apker, W. H. Brattain and C. Herring. Although the conference was nominally as international as those previously held at Reading, Amsterdam and Garmisch-Partenkirchen on the same subject, it was in fact dominated by American contributions and participation; but the importance which has long been attached to the subject by American defence departments, industry and universities assured an excellent array of papers, even though none seems destined to alter profoundly the course of the subject.

The exciton was given much attention. Broser, who had, two years previously, invoked exciton diffusion to account for the propagation of photoconductivity in some semiconductors, now considered that photon emission, scattering and reabsorption better explained the findings of further experiments. His earlier co-worker, Balkanski, on the other hand, did not reject the idea that excitons could have large diffusion lengths. Much interest was aroused by reports on clean surfaces of semiconductors (freshly cleaved or cleaned by bombardment), which require vacua of the highest order for their examination. At the same time, the practical importance of real surfaces was reflected in the continued efforts to find a model which explains the dependence of the surface electrical properties on the bulk properties, preparation and environment. Compound semiconductors, though not ignored, did not play a leading part; further physical investigations of some probably await a tenfold reduction in the content of the residual impurities. Hot carriers figured prominently in a session on transport phenomena, but the avalanche effect and tunnelling, which has more recently opened up a new field of application of degenerate semiconductors, were not mentioned.

If the report lacks a few outstanding papers, it none the less shows that further conferences can be held on this subject; the scope may have to be limited, however, if both the conferences and their reports are not to be unwieldy. J. R. Tillman

ENGINEERING STATICS

Elements of Engineering Statics

By Prof. H. Deresiewicz. Pp. vi+124. (New York: Columbia University Press; London: Oxford University Press, 1958.) 28*s*. net.

STATICS has gained importance in engineering science because, together with the geometry of deformation, it forms the basis of structural analysis generally. However, owing to the apparent requirements of engineering practice over the past 100 years, a variety of set procedures for the analysis of structures has been developed, using the principles of statics and geometry and the assumption of elastic behaviour. Accordingly, text-books on theory of structures have tended to be concerned more with the details of these procedures, or so-called methods of analysis, than with general principles. This has led to some confusion and misapplication of principles as well as failure to adopt a rational and discriminating approach to structural analysis. Thus, even in published work, the so-called principle of least work, due to Castigliano (1873), has been regarded as an application of the statical principle of minimum potential energy. While the former is concerned with the compatibility of deformations within statically indeterminate structures the elasticity of which is linear, the latter is concerned with the stable equilibrium of conservative statical systems generally.

Having regard to this background, a book of the kind written by Prof. Deresiewicz, which presents the principles of statics in relation to the requirements of engineering science, is welcome indeed. It contains chapters on the elements of vector algebra, equilibrium including consideration of statical determ-

inacy, equivalence of force systems, simple structures, sliding friction, work and energy methods and equilibrium of space systems. Chapter 7, on work and energy methods, is particularly important as an introduction to energy principles and their power as analytical devices, and should serve to prevent the kind of confusion to which reference is made above. Thus, sections on real and virtual work, potential energy and stability are included in this chapter.

Another important feature of the book is the large number of worked examples which it contains and the appended selection of examples for solution, with answers. It is to be hoped that this book attracts the attention of teachers and students and that, together with similar works in the future, it will lead to more advanced courses in applied statics to supplement those in techniques of structural analysis.

T. M. CHARLTON

GRASSLAND MANAGEMENT

Grass Productivity

By André Voisin. Translated from the French by Catherine T. M. Herriot. Conversion of Tables by M. M. Sandilands. Pp. xiv+353+41 photographs. (London: Crosby Lockwood and Son, Ltd., 1959.) 50*s*. net.

THIS book by André Voisin is timely if only for the influence it may have in re-stressing the importance of exploiting pasture by better grassland management practices. M. Voisin is a scientist who became a practical farmer; he farms a sixty-acre dairy farm wholly in grass in the Department of Seine Inférieure near Dieppe. He has clearly given much thought to the problems of growing grass and of using it intensively by means of his dairy herd. He is an enthusiast particularly in respect of the health of his animals and as a farmer is much concerned with high yields, attaining thereby a satisfactory economic return. He regards the process of grazing as the "meeting of cow and grass" and throughout his book indicates the importance of this concept by laying emphasis on the facts that the yield from grazed pasture enormously depends upon the way animals are put to graze it, and also on when they graze in relation to the physiological growth of the herbage offered them.

High quality and maximum yields from pasture lands in France, as in Britain, come about as a result of good farming practices and one feature of these is the part which animals play in maintaining the botanical balance between species in the pasture. It is by no means certain, however, that the animal is an essential ingredient of the 'high-production pasture complex'. Indeed, many pasture fields in Alpine Europe are highly productive by any modern standard and yet are seldom grazed by animals. The recent interest in so-called 'zero grazing' and the nineteenth century vogue for 'soiling' in Britain involve periodic harvests of herbage and feeding it immediately to animals living away from the field on which it is grown. The success of all these schemes depends upon maintaining rapid growth and high levels of herbage yields by fertilizer (including dung and urine) applications to the pasture fields. Given these conditions there need be no difficulty in maintaining either immediate yield or balance of botanical constituents upon which continued productivity depends. Success as a viable economic process depends much also upon the input of labour and machinery in relation to net output of animal product, compared with other systems of utilization.

M. Voisin attempts to describe in some detail the mechanism of grazing and perhaps draws too large a distinction between normal grazing by the cow and harvesting by machine. Undoubtedly there are differences between these two mechanisms, but it is dangerous to over-stress the differences out of context. It may be that the mechanics *per se* of grazing are no more important than is the fact that animals deposit dung and urine on the land from which they graze, and thereby return plant nutrients into circulation and so accelerate the rate of turnover of nutrients within the plant/animal/soil system. M. Voisin makes much of the concept that "cows refuse to work any hours of overtime". The suggestion that grazing animals feed when at pasture for strictly eight hours a day is based on quite fragmentary evidence. In fact there are a number of records in the literature which show that in many circumstances the ruminant will actively graze for many more hours a day than the stipulated 'union rate' of eight hours.

The book is excellently produced and the English translation done well, although a number of errors occur in the text. Some of these are almost certainly due to translation and can be eliminated in a second edition. There is also a good deal of repetition which is possibly more obvious in the English text than in the original. M. Voisin's book, however, should prove a useful addition to the library of any thinking husbandryman.

W. DAVIES

HUMANE EXPERIMENTS

The Principles of Humane Experimental Technique

By Dr. W. M. S. Russell and R. L. Burch. Pp. xiv+238. (London: Methuen and Co., Ltd., 1959.) 30*s*. net.

EXPERIMENTS on living animals present a number of difficulties. Individual animals differ from one another in their responses, so it is usual to make numerous replicate observations as a basis for reliable predictions. Animals are sensitive to small changes of many kinds in their environment, and this sensitivity can affect an animal so that it reacts differently to the same experimental stimulus on different occasions. The more disrupting the stimulation, the more violent the reactions of the animal and the more inconsistent its response. These are also the circumstances in which the animal is in all probability distressed or in pain. The various responses which are associated with distress and pain, and which help the animal to overcome or escape from injury greatly complicate any study of the direct response to the initial stimulation, and so are usually undesirable from the scientific as well as the humane aspect. Every step which can be taken to reduce the complexity of an animal's response will reduce also the experimental variation, and so there is good reason for considering humanity and experimental design as one problem.

It is both humane and economical also to use as few animals as possible in experiments. However, it is rather difficult to know to what extent in Britain the use of animals is minimized. The figures available from the Home Office returns are not

detailed enough to be informative. More extensive information was obtained in a survey conducted by the Laboratory Animals Bureau in 1952. This survey depended on the voluntary co-operation of the relevant laboratories, but as about 80 per cent replied, the results are reasonably representative. The material has already been analysed by Lane-Petter with particular reference to the procurement of experimental animals, and it is now analysed again in this book by Russell and Burch from the point of view of their use.

It must be admitted that it is a rather incoherent body of data and its defects are indicated (as Dr. Russell admits) when 108 canaries of Lane-Petter's report reappear in the present analysis as 72 ducks. However, it is the best available, and its inadequacy suggests that the best and most economical use of animal resources does not receive as much attention as it deserves. The rest of the book deals with this problem in terms of replacement of the whole animal by a physical or chemical system, a tissue culture, or a preparation of an isolated organ; reduction of the numbers needed by avoiding quantal observations and other extravagant designs and by good environmental control; and refinement of technique in choosing suitable species, responses and methods of approaching a problem.

All these approaches are desirable and useful, but must be considered in the light of the particular problem which it is necessary to solve experimentally. It is useful to have a résumé of ways which have already been adopted to make experimentation as humane as possible, and so this book is in some ways supplementary to the well-known UFAW handbook on the care of laboratory animals. It is not sufficiently informative to be used as a guide either to details of experimental design or to the husbandry of experimental animals. Perhaps its chief purpose is to stimulate thought on both these topics, and it is to be hoped that it will succeed in doing so.

M. Weatherall

U.S. ARMY NEUROSURGERY IN THE SECOND WORLD WAR

Surgery in World War II. Neurosurgery, Vol. I

Colonel John Boyd Coates, Jr., M.C., Editor in Chief (Medical Department, United States Army). Prepared and published under the direction of Major General S. B. Hays. Pp. xix+466. (Washington, D.C.: Government Printing Office, 1958.) 5 dollars.

A HISTORY of surgery in the Second World War is in course of preparation by the Medical Department of the United States Army. Two volumes are devoted to neurosurgery. The first of these has been published and deals with the organization of the neurosurgical services and the care of head injuries at all stages after wounding. The second volume will deal with spinal and peripheral nerve injuries.

The first volume is edited by Drs. R. G. Spurling and Barnes Woodhall, contains 466 pages, and is beautifully produced and illustrated. Although fourteen authors have contributed sections, there is little overlap between these, and the quality of almost every section is extremely high. The early chapters are concerned with the planning and development of neurosurgical services in the United States itself, and the European and Mediterranean theatres, and reveal what can be achieved by excellent organization. Thus the nine neurosurgical centres set up in the United States in August 1942, each with 150 beds, had increased in two years to nineteen, each with 350–500 beds, and within another year certain of these hospitals had 2,000 beds. Although a programme of training for military neurosurgeons was instituted early in the War, it is no surprise to hear that it was at times difficult to find what was considered to be the appropriate medical personnel for these numerous centres, in particular at the ward officer level. When the demand for neurosurgeons overseas developed, the problem of distribution of personnel was at times difficult. The assistance of organic neurologists would have been invaluable but their scarcity in comparison with neuropsychiatrists rendered such aid quite infrequent. The contributions of the consultant neurosurgeons in disseminating knowledge through visits, newsletters and conferences were invaluable. In all theatres of operations it was believed that neurosurgeons could be most profitably employed in evacuation hospitals 15–20 miles from the front, the patients being transferred to neurosurgical centres some days later.

The greater part of the volume is given over to the consideration of therapy in the head injuries of modern warfare, and contains several outstanding contributions. In one of these the modern management of missile wounds of the head receives detailed consideration. The post-operative fatality rate in several groups of patients treated by such techniques was approximately 14 per cent, another 12 per cent of the patients reaching hospital after a head injury dying without treatment. These figures give eloquent testimony to the success of the plans for treating neurosurgical casualties and the skill of those who carried them out.

Another excellent contribution deals with post-traumatic epilepsy. Almost 250 patients were submitted to a carefully planned study. In 55 per cent. seizures were controlled medically for a minimum period of four months; 17 per cent were lost to the investigation and in 28 per cent the attacks remained uncontrolled. Two-thirds of the latter group of 66 patients were treated surgically. From the early results it is considered that surgical measures may have a limited place in the management of post-traumatic epilepsy which does not respond to medical measures.

Other articles deal with pathological aspects of missile wounds, blast concussion, infective complications of brain wounds and ventricular penetration by missiles, as well as the repair of skull defects. Perhaps the only omission is a discussion of the rehabilitation and resettlement of patients with brain wounds. Such a section would have completed the story of the group of patients so fully considered from other points of view.

This contribution to the history of surgery in the United States Army in the Second World War is a very valuable one. It is a fascinating account of the development of the Army neurosurgical services and their utilization during the greatest of wars. Moreover, it provides in readily available form detailed information which will be invaluable to those concerned in the development of a military neurosurgical service in the future, should the need for this arise.

J. E. A. O'Connell

Précis de Pétrographie

Roches Sédimentaires, Métamorphiques et Éruptives. Par Prof. Jean Jung. Pp. 314+20 planches. (Paris: Masson et Cie, 1958.) Broché, 3,600 francs; Cartonné toile, 4,600 francs.

TO Prof. Jung, petrography and petrology are ill-separated, and in this book he considers not only the descriptions of different sorts of rocks in thin section but also their mutual relations and origins. The book is intended as an introduction to the science of rocks for students engaged on degree courses, and it is an admirable introduction, concise and essentially complete. There are, however, no references or bibliographies, which may be considered a drawback in a work which is so condensed. It is organized in four sections, the first on rock-forming minerals, the second on sedimentary and residual rocks, the third on metamorphic rocks, and the fourth on igneous rocks, each of which is copiously illustrated with excellent line drawings and photographs.

An early chapter contains one of the most compact, readable, logical, and memorable expositions of the structure of the silicates to be found in any text-book, and sets the tone for the rest of the book. The clarity of the writing is such that very modest linguistic ability, aided by a good dictionary, is sufficient to make the text easily intelligible. Even so, it is probable that the majority of English-speaking students will not wish to add translation to the other burdens involved in mastering a new subject, and possibly the greatest value of the "Précis de Pétrographie" outside the French-speaking world will be to more advanced students, who will find in it a rapid means of verifying the systems of rock nomenclature and classification in use in France, together with the accepted views on petrology current there.

J. C. M. TAYLOR

The Physics of Elementary Particles

By Prof. J. D. Jackson. (Investigations in Physics, No. 9.) Pp. x+135. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1958.) 30*s*. net.

THIS book gives a simple and readable account of the main features of elementary particles and their interactions other than the electrodynamic interaction of photons with electrons and positrons.

The first part of the book deals with pi-mesons and nucleons, particularly pion-nucleon scattering and photo-pion production. These processes are treated from a semi-classical point of view, but an outline is also given of the dispersion relation approach to scattering. The determination of the spin and parity of the pions is not discussed, which is a pity, as a few extra pages would have added greatly to the completeness of the picture.

The next section gives the basic facts about k-mesons and hyperons, introduces the concept of 'strangeness' and distinguishes clearly between strong and weak interactions.

The last part of the book describes the weak interactions, including a discussion of space and time reflexion, and charge conjugation invariance. The final sections review the experimental determination of the β-decay interaction and discuss the universal Fermi interaction.

In his preface the author wisely remarks: "In a field of physics that is changing every instant it is senseless to hope that a book written to-day will endure beyond to-morrow, but I hope that this volume will serve for a short time as a useful introduction to a fascinating subject". He has succeeded admirably in this limited objective.

P. T. MATTHEWS

Analytical Chemistry

Some New Techniques. By A. G. Jones. Pp. viii+268. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 40*s*.

IN this useful book, several of the more recent techniques of analytical chemistry are described. Larger texts, mainly American, have appeared which cover similar ground, but these have generally been designed for advanced student training. The book under review is aimed at senior chemists who have little time to delve into the original literature and who want a succinct account of a new technique.

The methods which have been chosen are flame photometry, differential spectrophotometry, gas chromatography, ion exchangers, acid-base titrations in non-aqueous media, coulometric titrations, differential refractometry and the determination of oxygen and hydrogen in metals. In each section, the principles of the method are delineated, the essential apparatus is described and the applications are discussed. A broad idea of the possibilities of each technique is given and, just as important, its limitations are mentioned; the reader is warned of the most likely sources of error. A number of references is given to more detailed texts and to the original literature.

The section on the determination of oxygen and hydrogen in metals is devoted to the analysis of the one-time rarer metals; most of the actual techniques in this part have been used for years in the iron and steel industry, but their broader application is quite recent and has involved special problems.

In general, this little book serves its purpose admirably and should be welcomed as a simple exposition of several very valuable techniques.

A. M. G. MACDONALD

Mites, or the Acari

By T. E. Hughes. Pp. vii+225. (London: The Athlone Press, University of London, 1959. Distributed by Constable and Co., Ltd.) 42*s*. net.

IT is surprising in view of the economic importance of mites and ticks that this should be the first comprehensive account of their biology and anatomy to be published in Britain. Mr. Hughes's text can be considered in two sections. In Chapters 1–6 an account is given of the biology of both free-living and parasitic species, while Chapters 7–14 deal with their anatomy. The formidable task of gathering biological information from a scattered literature and presenting a readable survey has been well done, although it is unfortunate that a number of errors have escaped the author's notice. For example, it is incorrectly implied that the Bdellidae are exclusively littoral and that the Rhodacaridae prefer saturated conditions. The second section is more authoritative. The discussion of the external and internal anatomy of the Acari is based on a critical review of evidence, a considerable amount of which has been derived from the author's own researches. The outline classification presented in the final chapter should prove useful to the general zoologist. The book is well produced and the text-figures are of high standard throughout.

G. O. EVANS

GAMMA-RADIATION FROM DEPOSITED FALL-OUT

By D. H. PEIRSON and L. SALMON

Health Physics Division, Atomic Energy Research Establishment, Harwell

THE rate of deposition of fission products from nuclear test explosions has increased since 1957. This increase is shown, for example, in the annual deposition of strontium-90 observed[1,2] in rainfall at Milford Haven, Pembrokeshire.

Table 1

Year	Deposition of strontium-90 (mc./km.²)
1952–54	2·0
1955	2·4
1956	2·5
1957	2·6
1958	5·4

About two-thirds of the deposition in the United Kingdom during 1959 is produced by the explosions of autumn 1958. This is deduced from measurements of the strontium-89/90 ratio in rainwater and similar data from dust samples from the atmosphere. Therefore, not only has the rate of fall-out increased but it also follows that current deposition contains a large proportion of fission products of half-lives shorter than those of strontium-90 (28 years) and cæsium-137 (30 years), which are considered to have the greatest long-term radiological significance. It is instructive to consider the increase of external gamma-radiation due to the deposition of the short-lived fission products.

Analysis of Soil

The increase in gamma-radiation has been analysed by assaying samples of soil and grass from several sites in the United Kingdom.

Samples of undisturbed topsoil, including vegetation and root-mat, are cut in dimensions 15 cm. × 15 cm. × 7 cm. deep. Each sample is thoroughly mixed and counted in bulk (~ 1 kgm.) by a gamma-ray scintillation spectrometer, containing a sodium iodide crystal (7·5 cm. × 7·5 cm. diameter) shielded by 10 cm. of lead and coupled to a multi-channel pulse analyser. The gamma-ray spectrum of the deposited fission products lies superimposed upon that of the natural radioactivity of the soil, namely, the uranium and thorium series and potassium. The spectrum of natural radioactivity is measured at each site in a second sample cut from immediately beneath the first. Comparison of the spectra from the two samples allows the contribution due to fission products to be extracted from the composite picture, since in all cases it is found that at least 95 per cent of the deposited fall-out is confined to the top 7 cm. of soil.

The principal fission products that have been assayed by this means in recent soil samples are shown in Table 2.

Parent and daughter activities are bracketed in this table. Half-lives and gamma-ray energies are available from Strominger *et al.*[3].

Table 2

Fission product	Gama-ray energy (MeV.)	Half-life
Cæsium-137	—	30 year
(Barium-137*m*)	0·661	2·6 min.
Zirconium-95	0·72, 0·76	65 day
Niobium-95	0·77	35 day
Ruthenium-103	0·50	42 day
Ruthenium-106	—	1 year
Rhodium-106	0·51	30 sec.
Cerium-141	0·145	33 day
Cerium-144	0·134	290 day

The results of soil assays at seven sites are summarized in Table 3.

Apart from the case of cæsium-137 the significant gamma-ray activities in the soil samples arise from combinations of pairs of fission products. The genetic relationship between zirconium-95 and niobium-95 permits calculation of the contribution from each, assuming the pair to be in transient equilibrium. No such relationship exists in the other pairs, so to avoid the uncertain conversion to beta-disintegrations, the surface concentrations of deposited fission products are expressed as mc./km.² of the appropriate gamma-disintegrations. An additional component observed in the spectra of most samples, but not listed in the table, has been attributed to zinc-65 (245 days). The contribution of this isotope to the total activity is insignificant.

Continuing deposition will increase and radioactive decay will decrease the content of any radionuclide. Since collection of the soil samples was extended over a period of three months, the activity of the short-lived gamma-ray emitters at the various sites should be compared in the light of these factors. The small contributions from cerium-141, -144, ruthenium-103 and rhodium-106 are a result of low yield per fission, low gamma-ray abundance per disintegration or previous decay. The activity of cæsium-137 represents an accumulation from explosions over some years, whereas that of zirconium-95–niobium-95 originates from the most recent series of tests.

Soil samples from Illinois in 1957 were analysed in a similar manner by Gustafson *et al.*[4].

Cumulative Deposition in Rain

The relationship between the surface concentration of cæsium-137 and mean annual rainfall is demonstrated in Fig. 1.

The concentrations of activity due to cæsium-137 and zirconium-95–niobium-95 at Milford Haven may be derived in another way by calculating the cumulative deposition, corrected for decay, in rainwater. These are plotted in Fig. 2. The activity of cæsium-137 was obtained from published deposition data[1,2]. Measurement of the deposition at Milford Haven of zirconium-95–niobium-95 (unpublished) started only in August 1957. An approximate

Table 3

Site and date	Cerium-141, -144		Ruthenium 103, Rhodium-106		Cæsium-137		Zirconium-95–Niobium-95		Total
	mc. γ/km.²	μr./hr.	mc. γ/km.²	μr./hr.	mc. γ/km.²	μr./hr.	mc. γ/km.²	μr./hr.	μr./hr.
Kew (Surrey) 26.8.59			8	0·07	13	0·15	34	0·44	0·7
Chilton (Berks.) 3.6.59			34	0·30	22	0·25	158	2·05	2·6
Abingdon (Berks.) 25.6.59	24	0·05	22	0·20	28	0·32	135	1·77	2·3
Milford Haven (Pembroke) 14.7.59	17	0·04	18	0·16	43	0·50	182	2·37	3·1
Eskdalemuir (Dumfries.) 28.8.59	19	0·04	27	0·24	54	0·62	130	1·00	1·9
Windermere (Westmorland) 10.7.59	24	0·05	33	0·30	75	0·86	331	4·30	5·5
Snowdon (Caernarvon) 27.6.59	40	0·09	57	0·51	133	1·53	567	7·38	9·5

starting point for this curve was obtained by the somewhat crude assumption that the cumulative deposition of zirconium-95 at this date was identical with that of strontium-89 (available from ref. 1). Any error due to this assumption becomes progressively less important after September 1957 on account of the radioactive decay. Thus by March 1958 the initial error has been reduced to one-tenth.

The calculation from cumulative deposition gives estimates of the concentrations of activity of cæsium-137 and zirconium-95 and niobium-95 at Milford Haven that are about 70 per cent of those from the soil assay. The factors that might contribute to this discrepancy include non-uniformity of the site drainage, inadequacy of the sampling method and the possibility of dry deposition. The analytical error, at least for the determination of cæsium-137 and zirconium-95–niobium-95, is probably not greater than 10 per cent.

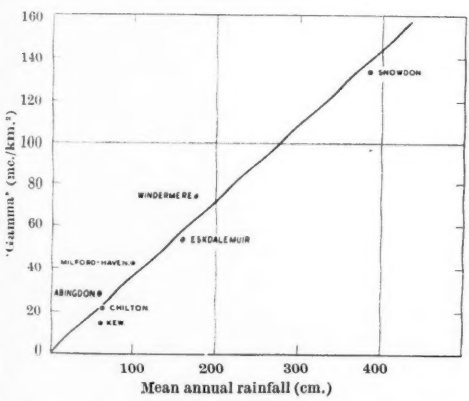

Fig. 1. Cæsium-137 in soil (June–August 1959) versus rainfall

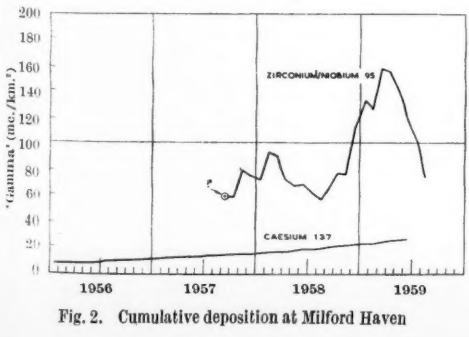

Fig. 2. Cumulative deposition at Milford Haven

Gamma Radiation Dose-rate

The surface concentrations listed in Table 3 are converted to gamma-ray dose-rates by using the data of Chamberlain[5]. The dose-rates at a height of 1 metre are calculated by neglecting absorption in the soil. The effect of absorption is small for the freshly deposited zirconium-95–niobium-95 but may be significant for the long-lived cæsium-137 that is subject to continuous weathering.

Only at the Chilton site is it possible to compare by direct measurement the gamma-radiation dose-rates due to fission product and natural radioactivity. Elsewhere no measurements were made before the period of significant fall-out. At Chilton the dose-rate due to natural radioactivity (excluding cosmic rays) is 5 μr./hr. The increase in June 1959 due to fission product deposition is ~ 50 per cent.

It is clear that, during this period of soil sampling, the enhancement of gamma-radiation dose was mainly due to the fission products zirconium-95–niobium-95. Undoubtedly, if nuclear test explosions were not resumed these short-lived fission products would become insignificant compared with cæsium-137. If the production of nuclear debris continues at the rate of 1957–58 then the gamma-radiation dose from zirconium-95–niobium-95 could become a significant radiobiological hazard. Thus at mean rates at Chilton corresponding to June 1959, the approximate annual dose above uncultivated land from zirconium-95–niobium-95 would be 20 mr./year and that for cæsium-137 2 mr./year, compared with about 40 mr./year from 'natural background' activity (excluding cosmic rays). At other sites having heavier rainfall and greater fall-out deposition the annual dose-rates would be correspondingly larger.

The dose to the population, making allowance for weathering and shielding by buildings, has been estimated by Spiers (see following article).

[1] Stewart, N. G., Osmond, R. G. D., Crooks, R. N., and Fisher, Miss E. M. R., A.E.R.E. Report *HP/R* 2354 (H.M. Stationery Office, 1958). Stewart, N. G., Osmond, R. G. D., Crooks, R. N., Fisher, Miss E. M. R., and Owers, M. J., A.E.R.E. Report *HP/R* 2790 (H.M. Stationery Office, 1959).

[2] Crooks, R. N., Osmond, R. G. D., Owers, M. J., and Fisher, Miss E. M. R., A.E.R.E. Report *R* 3094 (H.M. Stationery Office, 1959).

[3] Strominger, D., Hollander, J. M., and Seaborg, G. T., *Rev. Mod. Phys.*, **30**, 585 (1958).

[4] Gustafson, P. F., Marinelli, L. D., and Brar, S. S., *Science*, **127**, 1240 (1958).

[5] Chamberlain, A. C., A.E.R.E. Report *HP/R* 2606 (H.M. Stationery Office, 1958).

SOME MEASUREMENTS OF BACKGROUND GAMMA RADIATION IN LEEDS DURING 1955–59

By Prof. F. W. SPIERS

Department of Medical Physics, and M.R.C. Environmental Radiation Research Group, University of Leeds

GREAT interest centres on the extent to which the fission products from nuclear weapon tests add to the dose received by human beings from natural environmental sources. The extra radiation dose is contributed both by radioactive nuclides which become incorporated in the body and by penetrating radiations from fission products deposited on the ground. Estimates of the external fraction of the dose[1,2] suggest that it is smaller than that received from internal sources, but such calculations necessarily involve many assumptions. The radiation dose out-of-doors is calculated for a uniformly contaminated plane surface and assumptions are made concerning the leaching away of surface deposition by rain. In estimating the dose to the population further assumptions must be made as to the shielding afforded by houses and the fraction of the day spent out-of-doors.

A number of measurements of the total γ-ray dose-rate have been made in Leeds over the years 1955–59, and these, together with other observations now reported, indicate that over undisturbed grassland a considerable increase in dose-rate has occurred. Present information is insufficient to determine an over-all average increment for sitesi ndoors and out-of-doors but the increase will be much less than that over grassland; tentative estimates, however, suggest that it is probably greater than the dose-rate to body tissues from ingested cæsium-137[2].

Measured γ-ray dose-rates at points 1 ft. above the centres of two small lawns in Leeds are shown in Fig. 1. They have been made at varying intervals since July 1955, the first four measurements being made with Geiger counters and those since August 1, 1957, with a portable high-pressure ionization chamber. The two Geiger counter measurements *C* and *D* in 1957 were standardized by comparison with the calibrated high-pressure ionization chamber; the measurements in 1955 (*A*) and 1956 (*B*), made with different counters, were each linked to those in 1957 by the γ-ray responses to a standard radium source.

The high-pressure ionization chamber was used in these investigations because its sensitivity and stability of response enable the level of background gamma-radiation to be followed over a long period of time. The chamber was used under drift-rate conditions and the change of potential of the central electrode was measured by applying a balancing voltage to the grid of a miniature electrometer valve in a Townsend balancing circuit. Calibration was carried out in a large hall, under conditions of minimum practicable scatter, with sources of radium and iodine-131 giving known gamma-ray dose-rates[3]. The final calibration figure was derived on the basis that the local gamma-radiation could be regarded as approximately equivalent in quality to a mixed radiation, in which 70 per cent of the dose-rate was attributable to radium gamma-rays and 30 per cent to iodine-131 gamma-rays[4]. A drift-rate of 1 volt per min. on the central electrode then corresponded to a γ-ray dose-rate of 50·3 m.rad per year or 5·73 μrad per hour. The stability of response of the instrument is shown in Fig. 1 by the measurements made inside a heavily shielded steel cubicle where the radiation, being almost entirely cosmic, is independent of changes in the local gamma-radiation outside it. The measured response in the steel room has remained throughout at 0·50 ± 0·01 volt per min. when corrected for the small changes of cosmic-ray intensity with barometric pressure. Corresponding to this response within the steel shield, a drift-rate of 0·7 V. per min. has been subtracted from the outside readings to allow for the cosmic-ray contribution.

The local γ-ray dose-rate over lawn 1 (Fig. 1) shows a small rise during 1955–57, but, in addition to fluctuations which include the transient effect of the Windscale reactor accident[3], there is a marked increase from the autumn of 1957 to May 1959. There are fewer observations on lawn 2, but they show a very similar rise. The dotted curve in Fig. 1 gives the total deposition of strontium-90 at Milford Haven[5], which provides some indication of the total deposition of long-lived fission products. The surface gamma-radiation cannot be expected to follow this closely, but the increase in slope of the γ-ray curve is roughly in step with the increased deposition of strontium-90 since the beginning of 1958.

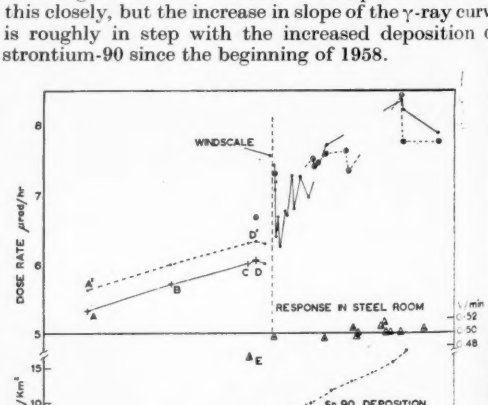

Fig. 1. γ-Ray dose-rates over lawns in Leeds; the line *A' D'* indicates the revised dose-rates for *A*, *B*, *C* and *D*, if the whole of the difference between point *E* (a single observation) and the other measurements in the steel room were attributable to grid current in the electrometer valve

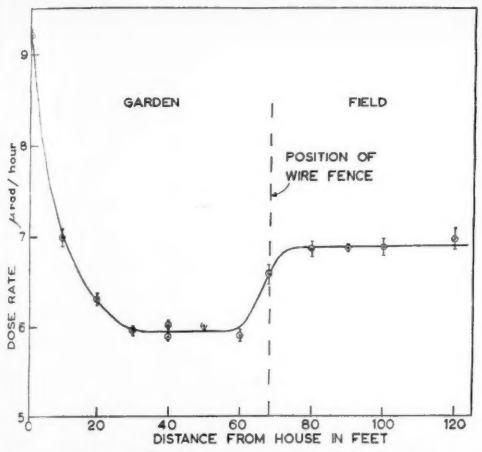

Fig. 2. Dose-rates over tilled soil and permanent grassland near Leeds, June 1959. (Tilled soil was 'rotovated' in December 1958, March and May 1959)

The most recent observations recorded in Fig. 1 (October 1959) show some decrease from the high values reached in May, in accord with the decay of the deposited zirconium-95–niobium-95 fission products which contribute a large fraction of the gamma radiation. The dose-rate over lawn 1 in May 1959 was about 3 μrad per hr., or 25 m.rad per annum, greater than in 1956. This measured increase can be compared with the dose-rates of 2·3–3·1 μrad per hr., calculated by Peirson and Salmon (see preceding article) from the measured amounts of fission products in the top 7 cm. of soil for sites at Chilton, Abingdon and Milford Haven. The increase in dose-rate measured at Leeds is about one-half that calculated for Windermere and one-third of that for Snowdon (Peirson and Salmon). Increments in the γ-ray dose-rate of 2·2, 1·2 and 3·1 μrad per hr. have been calculated by Gustafson[6] for the months of April, July and September 1958, respectively, on the basis of the measured fission product activity in soil at Argonne National Laboratory.

An observed difference in the γ-ray dose-rate over tilled land and permanent pasture is shown in Fig. 2. The dose-rate of 9·2 μrad per hr. near the house wall includes the contribution from the radioactivity of the brickwork, but at 40 ft. from the house the dose-rate at a height of 1 ft. is evidently representative of the activity of the surrounding land. The dose-rate over the uncut pasture is about 1 μrad per hr. greater than that over the garden, a difference which may be reasonably ascribed to burial of the fission products by mixing them to a depth of 6 in. to 9 in. by 'rotovation'. One month later, after the grass had been cut and removed, the difference between the field and the garden had fallen to about 0·5 μrad per hr. and 5 months later the dose-rates at the two sites were the same, within the error of measurement. Incidentally, at the time the hay was stacked the dose-rate near the haystack was 4 μrad per hr. above that in the open field.

The finding that radioactive deposition is located in the upper layers of undisturbed soil (Peirson and Salmon as ref. 6) is supported by some observations of the γ-ray dose-rate over a small lawn deliberately contaminated with iodine-131 at a level of 40 μc. per sq. m. This contamination produced an initial dose-rate (at 1 ft. above the lawn) of 105 μrad per hr., or about ten times the background-rate, on March 17, 1958, and the dose-rates, measured periodically over the next 45 days, are shown in Fig. 3. It was hoped that the rainfall of an English spring might provide evidence of the leaching effect of the iodine-131 from the soil. The rainfall was rather small, however, and the test fell short of its original design. It is evident from Fig. 3 that the dose-rate diminished at a rate almost equal to the iodine-131 decay rate and that the rainfalls of 1·2 in. for April and 0·7 in. for May did not appreciably affect the dose-rate by carrying the contamination to any considerable depth.

Although it is not possible to assess accurately the effect of fall-out on the external dose-rate to the population, it is useful to consider what increase in average dose-rate might be implied by the present observations on the Leeds lawns. With regard to weathering, the evidence on the deposition of fission products in the surface layers of undisturbed soil suggests that the dose-rates observed over lawns are not very much less than those to be expected if the fall-out remained without loss on the surface. An indication of the attenuation which might result from absorption of radiation, when the fall-out is distributed throughout the top 3 in. of soil, is given by observations of reduced dose-rate over snow. On two occasions in 1958, layers of snow about 4 in. deep reduced the observed dose-rates on lawn 1 to about 85 per cent of the 'unshielded' values obtained immediately after the snow melted. About the same degree of attenuation might reasonably be expected for the radiation from fission products lying at a mean depth of 1–1·5 in. The observed increase of about 25 m.rad per annum in the dose-rate over lawn 1 since 1956 might be taken, therefore, to imply an increment of about 30 m.rad per annum under conditions of no weathering or other removal by rain.

In deriving the population dose a factor for weathering must be introduced because washing away of fall-out is to be expected over streets and hard surfaces. Further factors for shielding when indoors must also be used. If, following the Medical Research

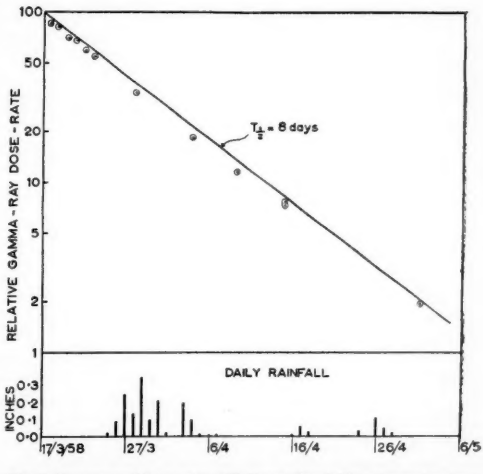

Fig. 3. Decrease of dose-rate with time after deliberate contamination of a lawn with iodine-131 to produce an initial dose-rate of about ten times the background-rate; daily rainfall given in inches

Council Report of 1956[1], we assume reduction factors of 3 for weathering and 7 for shielding, and an average period of 2·5 hr. a day spent in the open, the implied increase in the dose-rate to the population, above the 1956 level, would be about 2·5 m.rad per year. The Report of the United Nations Scientific Committee[2] discusses the range of weathering and shielding factors used by different authors and adopts a combined over-all factor of 10. On this basis, the increment of 30 m.rad per annum in the uncorrected outdoor dose-rate would lead to an increase of 3 m.rad per annum. Because of shielding of the gonads by overlying tissues, the increment in the average gonadal dose-rate would be about two-thirds of these estimated external rates or about 2 m.rad per year.

There are, however, considerable uncertainties as to the values to be used as representative weathering and shielding factors and at present the limited evidence we have collected is insufficient to establish reliably the average change in the dose-rate inside houses. At one site, in a ground-floor room facing lawn 1 in Leeds, an increase in dose-rate has been observed which amounts to about 80 per cent of the outdoor value. After the Windscale reactor accident, the increase in this room was one-third of the outdoor value and the higher fractional increase now obtained might be taken to indicate the presence of fission products indoors. In a single-story laboratory, the background dose-rate increased by 7·5 m.rad per annum between October 1957 and October 1958, with a further increase of 4 m.rad per annum by July 1959—increments which are about half those observed over lawn 1, but in step with them. In a second-floor room in another house no significant change occurred over the period June 1958 to June 1959, during which time the dose-rate over lawn 1 increased by about 5 m.rad per annum. An average shielding factor for the inside of a house, which depends on the siting of rooms, door and window areas and the presence of neighbouring buildings, is difficult to assess, but it may be less than the value 7 adopted above.

The present increase in the gonadal dose-rate may, therefore, be as little as 2 m.rad per annum, but this estimate is likely to be low rather than high. The increment, however, is greater than the values of 0·25–1 m.rad per annum suggested (so far for the year 1957 only) by the United Nations Scientific Committee[2] and it is greater than the average increment in gonadal dose-rate from ingested cæsium-137 which, in 1958, amounted to approximately 1 m.rad per annum[2]. A probable upper limit to the dose-rate increment can be suggested if the ground-floor observations are regarded as typical of the lower range of building protection factors. An average protection factor of about 2 is then implied, leading to an increment of about 4 m.rad per annum, if a weathering factor of 3 is used, and about 10 m.rad per annum in the unlikely event of no removal of fission products by rain. It is probable that the present increment in dose-rate to the population from external fall-out is not greater than 5 m.rad per annum. The external contribution to the fall-out dose-rate may be expected to decrease with the decay of the zirconium–niobium fission products if no further nuclear bomb tests occur.

[1] Marley, W. G., Medical Research Council Report on Hazards to Man of Nuclear and Allied Radiations (Cmd. 9780), 121 (London, 1956).

[2] Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, 101 and 109 (1958).

[3] Spiers, F. W., *Nature*, **183**, 517 (1959).

[4] Vennart, J., *Brit. J. Radiol.*, **30**, 55 (1957).

[5] Stewart, N. G., Osmond, R. G. D., Crooks, R. N., Fisher, E. M. R., and Owers, M. J., Report A.E.R.E. *HP/R* 2790, and personally communicated supplementary data. A.E.R.E.—*R* 3094.

[6] Gustafson, P. F., Argonne National Laboratory, Semi-Annual Report, *ANL*-5967 (July–December 1958).

[7] Spiers, F. W., Medical Research Council Report (Cmd. 9780), 111 (London, 1956).

ADDITIONS TO THE BRITISH MUSEUM (NATURAL HISTORY)

By Sir GAVIN de BEER, F.R.S.

Director

THE new premises recently taken into service in the Natural History Museum fall into two categories: those devoted to the Museum's major task of scientific research, and those devoted to the educational function of providing exhibits illustrating various aspects of natural history and of giving instruction. To the former belongs the new Mammal Building, erected to take the place of the old building demolished five years ago to make room for the new boiler house, which provides heating for the surrounding educational establishments in the South Kensington area.

Built to the plans of Mr. W. Kendall, the new Mammal Building stands 110 ft. high, consists of 11 floors, and provides 62,500 sq. ft. of accommodation for housing the national collections of mammals. Arranged systematically on each floor, the plan adopted provides for many years of expansion and acquisition of new specimens which will take their place in the collection without the necessity for removing other portions in order to make room. The importance of this provision is already seen in the efforts now being made to take advantage of the situation in Central and East Africa, where heavy casualties are inevitably being inflicted on large mammals as the result of policies that have been adopted. An opportunity exists which, it is hoped, will not be missed, to collect considerable quantities of skins and skeletons of ungulates, not only because adequately documented specimens of these animals are insufficiently represented in the existing collections but also because it is becoming increasingly important to determine the range of variation of species in Nature, and this necessitates possession of large numbers of specimens.

Preservation of mammalian collections presents many problems arising from the change in physical conditions of the atmosphere. Research is constantly proceeding into the most effective way of preserving skins, but no amount of precautions would be of any

avail unless two major variables of the atmosphere were not kept under control: humidity and temperature. The new Mammal Building is air-conditioned throughout to maintain the contents within prescribed limits of temperature and moisture.

Provision has been made for the accommodation of scientists, both members of the staff and visitors working on the mammal collections, and laboratories have been conveniently placed on the various floors of the building where the specimens can be adequately deployed and studied.

The new North Block of the Natural History Museum now accommodates the General Library and Map Room. The Library of the Museum is not a public library, but the working equipment of the scientific staff of the Museum to which qualified persons, both trained scientists and students, may be admitted on recommendation. The reading room, catalogue room, accessions room, studies for the librarian's staff, and book stores, conform to the most modern standards of equipment. Visitors who remember the cramped quarters in which the General Library previously had to carry out its functions will be agreeably surprised at the new library.

The Map or Atlas Room is a special feature of the General Library and is provided with ample accommodation for maps in shallow drawers containing a small number of maps each, so that any map can be easily and speedily extracted for study on the tables provided for their deployment. Adequate facilities for pin-pointing any place on the entire map-covered surface of the globe, both on dry land and under the sea, play an essential part in the equipment of the Natural History Museum for the work of every one of its five Departments. Gazetteers, books relating to topography and reports of scientific expeditions have been specially sited in the new Map Room, which also houses the large folio and double folio volumes too large for shelving elsewhere.

The new facilities provided for the discharge of the educational functions of the Museum comprise fresh exhibits and a Lecture Hall.

The fifth bay on the western or left side of the Central Hall of the Museum has been set up to illustrate the "Six Milestones of Evolution". These are: (1) The origin of life, illustrated by means of molecular models showing Miller's experimental production of the amino-acid glycine from hydrogen, water, carbon dioxide, methane, and ammonia, by means of electrical discharges and ultra-violet light. There are also models of adenosine triphosphate and of the Watson–Crick–Wilkins structure of deoxyribonucleic acid constructed on a scale of 2 cm. to the angström unit by Mr. A. H. Barker.

(2) The origin of plants and animals, illustrating the fundamental distinction in methods of nutrition between these two types of life and its consequences. An electron-microphotograph of the influenza virus by Dr. R. E. G. Wyckoff is also shown, together with examples of exceptions to the normal distinction in feeding mechanisms between plants and animals, such as parasitic plants and animals, and fungi.

(3) The origin of sex, traced from the simplest form of protistan cell undergoing fission and fusion without differentiation between the sexes, up to higher plants and animals where the specialization between mobile sperm and immobile food-containing eggs is reflected in all the differences between the individuals of the two sexes.

(4) The origin of the multicellular type of organization, of embryonic development, and of death form a compact subject of interrelated processes, illustrated with reference to the potential immortality of unicellular organisms, the mortality of the soma, and the development of the frog from the unicellular fertilized egg to the multicellular adult.

(5) The colonization of land by plants and animals, which introduced two important biological consequences: the alternation of generations in plants between gametophytes and sporophytes, and, in animals, the production of adaptations permitting eggs to be fertilized and developed out of water. These are illustrated by means of models and paintings of algae, mosses, ferns, and flowering plants on one hand, and fish, amphibia, reptiles, birds' eggs, and mammals on the other.

(6) Finally, under the head of psycho-social evolution, the briefest possible introduction is given to the phenomenon of non-genetically transmitted inheritance, instruction, and tradition. This is illustrated by means of experiments by Dr. W. H. Thorpe on the learning of the characteristic song by young chaffinches from adult chaffinches, pictorial representation of the customs of ancient Sparta, and of the cave art of palæolithic man, the snake of Aesculapius on a surgeon's glove, a microphone and printed page, and the shadows of the Houses of Parliament and St. Paul's Cathedral.

The exhibits were prepared and constructed by Mr. Erik Thorn.

The North Hall of the Museum has been reopened and contains two sets of popular exhibits which have, unfortunately, had to be withheld from the public for some time. One of these is the series of British trees, showing their appearance in summer and winter, with specimens of leaf, bark, flower, fruit and seed. The other consists of the series illustrating the importance of accurate identification of organisms for the efficient control of pests, plant and animal. An old friend exhibited here is the enlarged model of the flea.

Of the domestic animals previously exhibited in the North Hall, the skeleton of 'Brown Jack' and the mounted skin of 'Mick the Miller' are now on view in the Mammal Balcony. The remainder of the collection of domestic animals, greatly in need of augmentation, has been transferred to Tring Museum, where it is hoped at some time to establish displays of the breeds of domestic animals.

From the North Hall a corridor leads to the Lecture Hall. Along the corridor are showcases containing selected large mammals, such as the grey seal, reindeer, lion, cheetah, leopard, jaguar, Malay tapir, yak, and two species of zebra. In each case, following current museum practice, a map is provided showing the geographical distribution of the animal.

Between the entrance doors of the Lecture Hall is an exhibit demonstrating steps in the mounting of mammals for exhibition prepared by Mr. A. G. Hayward, senior taxidermist in the Museum. It is sincerely to be hoped that this exacting scientific and artistic technique will never again be referred to as 'stuffing an animal'. Facing the entrance to the Lecture Hall are some showcases devoted to the exhibition of notable paintings and drawings illustrating natural history.

The Lecture Hall is provided with the most modern equipment for projecting slides and films, and for satisfactory acoustic conditions. It accommodates 220 persons, and is used for lectures by the staff of guide-lecturers of the Museum and on other occasions.

OBITUARIES

Dr. D. O. Morgan

Dr. Daniel Owen Morgan, of St. Catharine's College, Cambridge, and University lecturer in animal pathology, formerly senior lecturer in zoology, University of Edinburgh, died in Cambridge on November 17, aged sixty-six. He was born on August 19, 1893, was educated at Tregaron County School, and after serving as a signaller during the First World War entered the University College of Wales, Aberystwyth, in 1919, reading zoology and agriculture and specializing in helminthology. His postgraduate researches were conducted under the supervision of Prof. R. T. Leiper, at the London School of Hygiene and Tropical Medicine, and included studies on the systematics of the trematode family Opisthorchidae, the bionomics of the potato eelworm *Heterodera schachtii*, and a survey of the helminths of domestic and wild animals in Hertfordshire.

During the years 1923–33 he held the post of field officer and senior research assistant at the Institute of Agricultural Parasitology of the London School of Hygiene and Tropical Medicine, and during this time published twenty-three papers on parasitological subjects. In 1933, he was appointed to a senior lectureship in zoology in the University of Edinburgh and became responsible for the teaching of helminthology to veterinary undergraduates reading for the Royal College of Veterinary Surgeons diploma and the University degree in veterinary science. In addition, he was responsible for the postgraduate courses in helminthology for the diplomas of tropical veterinary medicine, tropical medicine and public health, while during the War an emergency short course in helminthology for medical officers was his concern.

Despite an extensive teaching programme he found time for research, and his outstanding contribution was the study of the helminth population of Scottish hill sheep and the discovery of the 'spring rise' phenomenon of worm egg output in these sheep. His work on hill sheep has stimulated numerous researches on this and allied problems both in Great Britain and throughout the world.

In 1952 he moved to Cambridge and was responsible for the course in parasitology to veterinary students. He greatly loved Cambridge, its life, and was particularly fond of his College, St. Catharine's. He was a man of abounding kindness and encouragement, and will be remembered by the hundreds of students whom he guided and sadly missed by his colleagues.

E. J. L. Soulsby

Mr. William Dallimore, I.S.O., V.M.H.

The death of William Dallimore took place suddenly at his home near Tonbridge, Kent, on November 7, in his eighty-ninth year. He entered the Royal Botanic Gardens, Kew, as a young student in 1891 and his official connexion with this institution extended over forty-five years. His earlier years at Kew were spent mainly in the Arboretum, of which he later had charge. It was this close association with a rich collection of living plants in his early years that enabled Dallimore, with his keen powers of observation and a good memory, to build up for himself his great knowledge of trees and shrubs which he was to put to such good effect in later years. He was one of Britain's leading authorities on trees and shrubs for half a century, having maintained his keen mental powers to the full until the end of his long life. He devoted special attention to the conifers, and his "Handbook of Coniferæ" (written in collaboration with the late A. Bruce Jackson), which first appeared in 1923, has remained a standard work.

Another of Dallimore's notable achievements was the tremendous part he played in starting and developing the National Pinetum at Bedgebury in Kent, a joint undertaking on the part of Kew and the Forestry Commission, commenced in 1925. The fine specimens of pines and other conifers now to be seen at Bedgebury constitute a fitting, living memorial to him. He supervised the raising of these trees from seed in many instances. One of the main thoroughfares in the Pinetum has, in fact, been named Dallimore Avenue. On his retirement from Kew in 1936, Dallimore moved to Kent to live, and continued to act in an advisory capacity in connexion with Bedgebury almost to the time of his death.

At Kew, Dallimore ceased to have charge of the Arboretum in 1909 when he was transferred to the Museum staff, his first task being to initiate and build up a museum of British forestry (now the Wood Museum). His knowledge of arboriculture and forestry was of great assistance to him in this. He became keeper of the Museums of Economic Botany in 1926. His published works included two other books, one on pruning and one on holly, yew and box; also numerous articles in horticultural, botanical and forestry journals.

The Kew Guild, an association of past and present Kew men, came into being in the early 1890's, largely as a result of Dallimore's initiative and energy. He himself became president of the Kew Guild in 1926. Dallimore had other interests besides plants. For many years he was an enthusiastic bowls player. He was also churchwarden at the Kew parish church. With his keen sense of humour and his amusing narratives he was always a popular figure. His two daughters survive him.

F. N. Howes

Mr. A. W. Siddons

In no subject of the curriculum has the method of teaching undergone greater change in the past fifty years than in mathematics. Before the turn of the century, the school course contained little more than arithmetic, manipulative algebra and the rote learning (sometimes even in Latin) of Euclid's proofs. The revolt was led by Rawdon Levett, mathematics master at King Edward's School, Birmingham, who, through a letter in *Nature* of May 26, 1870, pleaded for the formation of an Anti-Euclid Association. It was left to one of his greatest pupils, Arthur Warry Siddons, to see the change effected, Euclid's stranglehold broken and geometry established as a suitable tool for school use.

Mr. Siddons devoted his whole life to school mathematics, though he might well have attained

eminence in the university field. He was a scholar of Jesus College, Cambridge, and was fifth Wrangler in 1898, immediately below Jeans and Hardy. In the following year he read natural science, and again took a first. His only appointment was at Harrow School; here he taught mathematics with increasing brilliance and success until his retirement in 1936, and in these years he earned the affection of generations of Harrovians. In 1902 Jesus College elected him to a fellowship and, in December of the same year at the age of twenty-six, he was appointed to a University Syndicate to discuss mathematical requirements. The report stated that any geometrical proof would be accepted provided it appeared "to form part of a systematic treatment of the subject"; Levett's cherished freedom was achieved. From this time to the end of his busy life, Siddons was engaged in converting the rigid logic of mathematics into a form suitable for young minds. In this his greatest collaborator was Charles Godfrey; as a team they had the ability to put into practice their progressive ideas and, when necessary, to write the appropriate text-books.

In addition to an intensely happy family life and to his devoted service to Harrow, Siddons's great joy was his work with the Mathematical Association. In 1902 he was elected secretary of the newly formed Teaching Committee. The reports prepared by this Committee now cover the whole field of school mathematics, and in preparing many of them, Siddons (and his life-long friend, C. O. Tuckey) took a leading part. The Association recorded its gratitude by electing him as president in 1935—he was the first schoolmaster ever to achieve this distinction—and in 1958 it bestowed the rare privilege of honorary membership.

With all his ability, all this distinction, Siddons was a very simple person. He was a great conversationalist and a most lovable man: in the Harrow tradition he was very fond of singing, and the School on the Hill has cause to remember his genius as a gardener and maker of lawns. He claimed that in his school-days he found mathematics difficult; perhaps this explains his success as a teacher and his passion for simplicity within the limits of exactness. To the end his mind was crystal clear, and he died peacefully in his sleep on November 10. Those who knew him well will miss his warm friendship, and the whole body of teachers and taught in mathematics is grateful for his life and his labours.

W. J. Langford

NEWS and VIEWS

International Scientific Relationships: Dr. Detlev W. Bronk, For.Mem.R.S.

A special medal (Fig. 1) has been awarded to Dr. Detlev W. Bronk, president of the Rockefeller Institute, by the Society for Promoting International Scientific Relationships. The award has been made for Dr. Bronk's accomplishments in fostering the promotion of scientific research and international co-operation through exchanges of scholars. The medal was presented on November 24 by Dr. Herman F. Mark, honorary president of the Society and director of the Institute of Polymer Research at the Polytechnic Institute of Brooklyn. At the meeting Dr. Alan T. Waterman, director of the National Science Foundation, delivered an address on "Science and the Progress of Man".

Fig. 1

Bristol City Museums: Dr. F. S. Wallis

Dr. F. S. Wallis will bring to a close his long and distinguished service to the Bristol City Museums in May of next year. A Bristol man, he was educated at the Fairfield Secondary School, Bristol, and at the University of Bristol, where his academic career was broken by service in the First World War with the Royal Warwickshire Regiment and the R.F.C., later the R.A.F. He graduated with first-class honours in geology in 1920, and afterwards took his M.Sc., his Ph.D., and finally, in 1930, his D.Sc. He was appointed assistant curator in geology after his first graduation and has devoted his life to the welfare and development of the Bristol Museum and Art Gallery, of which he was promoted to be deputy director in 1930. On the division of the Museum and Art Gallery in 1945, he became director of the City Museum—now 'Museums' as they include the Blaise Castle Folk Museum. He has never forgotten his first love as he has held for more than thirty years the post of special lecturer in sedimentary petrology in the University of Bristol and has since 1936 taken the evening tutorial class in geology there. He has always been interested in adult education and has lectured extensively to members of various organizations in Bristol and the surrounding district. For his field-work he was awarded the Lyell Fund of the Geological Society. In the museums field, he has long laboured for better lay-outs, more didactic exhibits and more artistic displays, and he has not spared himself in extending good ideas and projects of reorganization throughout the museums of the south-west of Britain. He served with unassuming distinction as president of the South Western Museums and Art Galleries Association and also, in 1956, of the national body, the Museums Association, in which he now ranks as an elder statesman. He gave notable service to the Joint Committee of the Museums Association and the Carnegie United Kingdom Trustees, both as a member and for five years as its chairman, in the worthy task of allocating grants for the reorganization of smaller provincial museums. He was war-time editor of the *Museums*

Journal, which did so much to maintain standards and morale at home, to hold and strengthen links with museum men overseas, and to build soundly based plans for museums in the post-War world. It is good that he is to continue to serve his chosen profession as curator of the museum of the Torquay Natural History Society.

Mr. Alan Warhurst

Mr. Alan Warhurst, who succeeds Dr. F. S. Wallis as director of the Bristol Museums, was educated at the Canon Slade Grammar School, Bolton, and the University of Manchester, where he secured honours in history, specializing in the Roman occupation of Britain. He is particularly interested in medieval archæology in its broadest sense and has undertaken field-research in Chester, Kent and Northamptonshire, having published a number of valuable papers. During the War he saw service in India, being commissioned to the Lancashire Fusiliers. His museum service began at the Grosvenor Museum, Chester, then extended to the Maidstone Museum and Art Gallery, where he was deputy curator, and in 1955 to the Northampton Museum and Art Gallery, to which he was promoted curator. There he started a major reorganization scheme and, having completed with notable success the archæological gallery, he was about to commence the boot and shoe gallery.

U.S. Scientific Attaché in India: Prof. E. C. Watson

Prof. E. C. Watson, dean of the faculty for the past fourteen years at the California Institute of Technology, is retiring to become scientific attaché to the U.S. Embassy in India. Dr. Watson administered the Institute's rocket development programme during the Second World War after having managed in the earlier days the Institute's physics laboratories. He has won national recognition for his studies in the history of science and for his remarkable collection of manuscripts, pictures and prints on the subject. He has published nearly eighty articles in the American *Journal of Physics* depicting the history of physics. This is in addition to numerous scientific papers and a book entitled "Mechanics, Molecular Physics, Heat and Sound". His main research has been in the field of X-ray photo electrons.

National Science Foundation : Dr. Bowen C. Dees

Dr. Bowen C. Dees has been appointed assistant director for scientific personnel and education at the U.S. National Science Foundation. Prior to going to the Foundation in 1951, Dr. Dees served as division chief and physicist with the Scientific and Technical Division of the General Headquarters of the Supreme Commander for the Allied Powers in Tokyo during 1947–51. He had previously lectured on physics at New York University, Mississippi College, the Massachusetts Institute of Technology and Rensselaer Polytechnic Institute. Dr. Dees received his A.B. from Mississippi College in 1937 and his Ph.D. from New York University in 1942.

Nomenclature in Titrimetric Analysis

During the Twentieth Conference of the International Union of Pure and Applied Chemistry, held in Munich in August 1959, the Committee of the Analytical Chemistry Section discussed certain points of confusion in the literature. Referring to the notation of indicators in titrimetric analysis, it was pointed out that many dyestuffs recommended as indicators in chelatometric and redox titrations have been given various trivial names. Examples can be found in the literature of a compound being recommended as a new indicator when the compound has in fact been in use for years under a different name. To avoid future confusion, the Committee recommends that when a dyestuff is proposed as an indicator in any type of titration, and particularly when it is claimed as new, the British Colour Index or Schultz Number be quoted in addition to the trivial name. As regards the terminology of complexing agents used as titrants, it is recommended that : (*a*) the group of polyamino-polycarboxylic acids which form anionic complexes shall be termed 'complexans' ; (*b*) titration processes in which any type of complexing titrant is used shall be termed 'complexometric titrations' ; (*c*) those complexometric titrations which involve titration with a chelating agent shall be termed 'chelatometric titrations' and represent a special type of complexometric titration.

Research Vessel for Woods Hole Oceanographic Institution

A new research vessel will be provided for studies in oceanography by a grant of 3,000,000 dollars from the National Science Foundation to the Woods Hole Oceanographic Institution. The new vessel will replace the research vessel *Atlantis*, the twenty-eight-year-old 'flagship' of the Institution's fleet. Because of the probability that the National Science Foundation would be asked to support the construction of research vessels, it made a grant to Woods Hole in 1958 to study capabilities and preliminary designs of various sizes and types of such vessels. This study, in turn, benefited from earlier studies made by the Institution with funds provided by the Office of Naval Research. The vessel will have an overall length of 175 ft., beam of 36 ft., and loaded displacement of 1,040 tons. She will have an operating range of 7,500 miles at a cruising speed of 12 knots. Incorporated in the design are roll- and pitch-damping devices, wide speed control and high degree of manœuvrability, superior laboratory spaces, and excellent working facilities. Preliminary design plans were prepared for the Institution by M. Rosenblatt and Son, of New York. Additional features which have been considered for possible inclusion in the detailed plans and specifications include anti-rolling tanks, acoustical 'quietness' for studying underwater sound characteristics, a gravity laboratory, an aquarium, and ample freezer space for preserving bottom-sediment cores and biological specimens. The total complement of the ship will be 37, of whom 19 will be scientists.

Radiocarbon Dating

The appearance of Radiocarbon Supplement Vol. 1 of the *American Journal of Science* marks an important step forward in the publication of radiocarbon dates. In the past, date lists have appeared at irregular intervals in a number of journals, making it difficult for potential users of the dates to keep themselves fully informed of all the work in this field. The dating laboratories have been well aware of this problem, but there are considerable difficulties in attempting to use any one of the existing scientific journals with a wide circulation. For this reason, the editors of the *American Journal of Science* are to be commended on this project, which provides a single, relatively inexpensive, annual publication specifically for radiocarbon dates and associated measurements.

This first volume contains thirteen date lists and one paper (Lamont VI) devoted entirely to measurements on samples of known age. This aspect of radiocarbon dating research has assumed considerable importance in recent years, for it yields information on the past and present distribution of radiocarbon in the carbon exchange reservoir, and this is of particular importance when one is concerned with the attainment of the highest possible accuracy in radiocarbon dates.

In addition to these contributions from dating laboratories, there is a very useful bibliography by Frederick Johnson, which gives references to all previous date lists, and a list of all laboratories known to be or to have been concerned with radiocarbon dating measurements. Thirty-six laboratories are listed, and it might be wondered why only about a third of them have contributed to this new publication. However, if one takes into account the fact that some have now ceased to be active in this field and that others have only recently begun operating, the response to this new project is not unsatisfactory. The editors have attempted to secure a high standard of presentation by requiring contributors to adhere as closely as possible to a set style and to include much informative detail regarding each sample in addition to its laboratory number and radiocarbon age. In general, the various authors have responded well to these requirements, and the standard achieved in this first volume promises well for the future. The Supplement is to be published annually, in May, at Sterling Tower, Yale University, New Haven, Connecticut; subscription rate 4.50 dollars a year; Vol. 1 will be sold for 2.50 dollars until January 1, 1960.

Inland Waterways in Britain

An interesting feature of the sixty-fourth annual report of the National Trust, for 1958–59 (London: National Trust), which records a welcome increase in free legacies and donations, is the reference to negotiations with the British Transport Commission for the acquisition of the southern section of the Stratford-upon-Avon canal and the southern section of the Staffordshire and Worcestershire canal. Since then it has been announced that, under an agreement to be approved by Parliament, the National Trust will lease the first of these canals for a trial period of five years, when it can be transferred outright or returned to the British Transport Commission. For the five years the National Trust will receive £1,500 a year from the Commission and the Trust will be able to provide in the towpath a thirteen-mile traffic-free walk through a remote and interesting countryside. Unless transferred to the Trust, this canal will be abandoned (at an estimated cost of £120,000) and the pleasures of fishing and boating on it would also be lost to the community. The second canal is of extreme beauty and importance and is James Brindley's earliest canal with locks. The Trust already owns property on this canal at Kinver and Wightwick. These transfers, which are foreshadowed in the Bowes Report on Inland Waterways, represent a new departure, both for the Trust and the Commission, and the legal and financial arrangements are not expected to be completed simply or rapidly. The report pays tribute to the help given by voluntary workers in scrub clearance, but, although the membership over the past fourteen years has risen from 10,300 to 80,668, many more members are needed if membership is fairly to reflect the wide responsibilities of the Trust and to support the extension of its work.

Newspaper Trusts

In Broadsheet No. 435, "The Work of Newspaper Trusts", Political and Economic Planning provides a concise account of the various newspaper trusts, their purpose and character and organization, with a view of assisting the assessment of their performance (*Planning*, **25**; 24 August 1959. London: Political and Economic Planning, 1959. 3*s*. 6*d*.). The fullest accounts given are of the Scott Trust, holding all ordinary shares in the Manchester Guardian and Evening News, Ltd., and the Observer Trust, but there are also notes on the provisions in the articles of association of *The Times*, *The Economist* and *The Spectator*. The Broadsheet points out that the organization of such a trust must be devised for the end to be served, and that many different methods may be adopted. Too precise a definition of a trust's aims or ideals could be harmful if circumstances changed. To form opinion and build good traditions and to appoint men of good sense to positions of responsibility are all that can wisely or legitimately be done to control the future. A newspaper trust is a novel but long-dated attempt to keep a newspaper in conformity with an existing ideal over future generations and it is still too early to judge how far these experiments have contributed to maintain the health and standards of the Press. It remains to be seen whether trusts can be more successful than other forms of proprietorship and control in preserving and developing the independence and character of a newspaper and simultaneously in achieving sufficient success to stay in business, if not to lead the field.

British Museum (Natural History)

Among the more important and interesting items reported to the Trustees of the British Museum at their meeting at the British Museum (Natural History) on November 28 was that Dr. G. H. Francis and Dr. A. J. Charig had returned from their expedition to South Peru. This expedition, the second from the Museum to this area, had for its objective the further study, collection and mapping of the rocks of the Province of Carabaya, in the Department of Puno, together with fossil collecting from new sites in Carabaya. The mapping, field geology and detailed study of igneous rocks were in the charge of Dr. Francis, of the Department of Mineralogy, continuing the work begun on the previous expedition which he led in 1954. The fossil and anthropological collections were made by Dr. Charig, of the Department of Palæontology. They were assisted by Messrs. S. G. McH. Clark and B. L. Holt, University of Cambridge, and by Sr. H. Salazar Dias, University of Arequipa. The expedition was in the field from June 1 until August 1, 1959. More than four hundred specimens of rocks and minerals were collected in the present field season. The survey of the area was continued, and a substantial number of fossils were assembled. In addition, the expedition brought home some anthropological material from Inca burials. Mrs. Rees Thomas and Lady Barlow have lent to the Museum a letter from Charles Darwin, dated July 5, 1844, asking his wife to arrange for the publication of his Essay on Transmutation of Species in the event of his death. The latter has been placed on exhibition in the case of Darwin relics, now on view in the Central Hall of the Museum.

Tanganyikan Stratigraphy

An authoritative and up-to-date account of the stratigraphy of Tanganyika is now available in English as a result of the work of members of the Tanganyika Geological Survey (Memoir No. 1: "Summary of the Geology of Tanganyika, Part 1: Introduction and Stratigraphy", by A. M. Quennell, A. C. M. McKinlay, and W. G. Aitken, 1956; 264 pp., The Government Printer, Dar es Salaam, 20*s*.). This memoir was prepared in draft in 1956 and used as the authority for the preparation of Lexique Stratigraphic International, Vol. 4, "Afrique", Fasc. 8c, "Tanganyika" (1957), but has only recently become available owing to delays in publication. Important revisions to the pre-Karroo stratigraphy have been made. The basement complex has for some time been known to contain rocks of different origins and ages in different areas, and several attempts at subdivision have been made as the structural and metamorphic history of the country has become better known. On the basis of the existing evidence, the present authors have adopted a five-fold subdivision, the systems being named in order of decreasing age the Dodoman, Nyanzian, Kavirondian, Usagaran and Ubendian, Karagwe-Ankolean, and the Bukoban Systems. The Dodoman System is correlated with the oldest known rocks of Africa, and the uppermost Bukoban is thought to be possibly Eocambrian.

The Institute of Metals

The following members have been elected to fill vacancies on the Council of the Institute of Metals with effect from the annual general meeting on March 29, 1960: *President*, Sir Ronald Prain, chairman and president, Rhodesian Selection Trust, Ltd.; *Vice-Presidents*, Dr. H. M. Finniston, research manager, C. A. Parsons and Sons, Ltd., and H. W. Hignett, assistant managing director, Henry Wiggin and Co., Ltd.; *Ordinary Members of Council*, Dr. R. W. K. Honeycombe, professor of physical metallurgy, University of Sheffield; Dr. Ivor Jenkins, chief metallurgist, Research Laboratories, General Electric Co., Ltd.; E. Robson, managing director, Manganese Bronze and Brass Co., Ltd.; J. Salter, director, British Aluminium Co., Ltd., and C. Smith, works director, James Booth and Co., Ltd.

Physical Society Exhibition

The Physical Society Exhibition will be held at the Royal Horticultural Society's Halls, Westminster, London, S.W.1 (just behind Victoria Street from Artillery Row), from January 18 until 1 p.m. on January 22. The opening ceremony will be performed in the New Hall on January 18, 1960, at 11 a.m., by Mr. A. J. Philpot (chairman of the Exhibition Committee and vice-president of the Physical Society) in the unavoidable absence of the president of the Physical Society. Lectures will be given by Flight Lieut. J. Billingham (R.A.F. Institute of Aviation Medicine) on "Some Reactions of the Human Body to the Stresses of High Performance Flight" on January 18; Dr. L. Essen (National Physical Laboratory) on "Atomic Time"; and Dr. D. A. Wright (Research Laboratories of the General Electric Company, Ltd.) on "Recent Advances in Solid State Physics". The lectures will be at 5.45 p.m. Further information can be obtained from the Society, 1 Lowther Gardens, Prince Consort Road, London, S.W.7.

University News: Birmingham

The title of reader in mathematical physics has been conferred on Dr. J. G. Valatin. Dr. D. A. Bell, reader in electromagnetism, and J. T. Allanson, senior lecturer in electrical engineering, have been appointed joint acting heads of the Department of Electrical Engineering until September 30, 1960, in the absence of Prof. D. G. Tucker, who is on study leave. The University has accepted the offer of Joseph Lucas, Ltd., to finance a Fellowship in the field of metal joining in the Department of Industrial Metallurgy for a period of seven years. Research fellowships have been awarded as follows: Dr. Florence J. Pepper (zoology); Prof. L. P. V. Johnson (genetics); M. J. Harrison (physics); and H. Mueller (biochemistry). The following appointments are also announced: Dr. H. B. Griffiths, to be lecturer in pure mathematics from July 1, 1960; and Dr. J. K. Brown, to be lecturer in chemistry from January 1, 1960.

Oxford

The following research grants are announced: from British Petroleum Trading, Ltd., £3,000 spread over three years for the purpose of continuing a research fellowship in geochemistry; and from the du Pont Company, U.S.A., 3,000 dollars for the year 1960–61, for the support of basic research in the field of theoretical chemistry in the Institute of Mathematics, under the direction of Prof. C. A. Coulson.

Announcements

Prof. E. G. Cox, professor of inorganic and structural chemistry in the University of Leeds, is to succeed Sir William Slater as secretary of the Agricultural Research Council on Sir William's retirement next year. Prof. Cox's appointment will take effect from July 1, 1960.

The eleventh Annual Lecture of the Plastics Institute will be delivered by Lord Verulam, who will speak on "The Brink of the Unknown". The meeting will be held in the Reardon Smith Lecture Theatre, National Museum of Wales, Cardiff, on April 7, at 6.30 p.m.

A symposium on Organic-Phosphorus Compounds will be held on January 29 in the Department of Chemical Technology, Bradford Institute of Technology. Those taking part include Dr. B. C. Saunders (University of Cambridge), Dr. D. J. Tuck (University of Nottingham), Dr. C. B. Reese (University of Cambridge), and Dr. A. F. Childs (research director, Albright and Wilson (Mfg.), Ltd.). Further particulars can be obtained from the Registrar, Institute of Technology, Bradford 7.

The Non-Destructive Testing Group of the Institute of Physics will hold a meeting in London during May 2–4 jointly with the Société Française de Métallurgie. The programme will have the general theme of the relationship between structure and physical properties of materials and will include recent advances in non-destructive testing techniques. Further information can be obtained from the Secretary, Institute of Physics, 47 Belgrave Square, London, S.W.1.

Erratum. We regret that in the article entitled "Counter-current Distribution of Proteins in Aqueous Polymer Phase Systems" in *Nature* of November 7, p. 1465, the blocks for Figs. 2 and 3 have been interchanged; the legends, as printed, stand.

INTERNATIONAL SYMPOSIUM ON MACROMOLECULES, WIESBADEN

THE annual International Symposium on Macromolecules, held under the auspices of the Commission on Macromolecules of the International Union of Pure and Applied Chemistry, this year took place in Wiesbaden, Germany, during October 12–17. Almost 1,200 people registered for the Conference, which must have been the largest of this series ever held. About half the delegates were from Germany and the remainder came from twenty-four other countries, with France, the United States and Great Britain supplying the largest delegations.

Organization of a symposium on the above scale must obviously be highly efficient if the work of the Conference is to be carried out, and the organizers deserve the highest credit for the arrangements they made. Meetings were held mainly in two large halls in the magnificent new Rhein-Main Halle, the acoustics of which were excellent. The general plan of the meetings was as follows. The Symposium was divided into five sections: (1) Physics of Macromolecular Substances; (2) Macromolecular Substances in Solution; (3) Elementary Processes and Kinetics of Polyreactions; (4) Chemistry of Organic and Inorganic Macromolecular Substances; and (5) Macromolecular Natural Substances and Model Compounds. Every day, a plenary lecture was given on some topic from one of the five sections. For the remainder of the morning, sessions of two sections were held simultaneously, at each of which three lectures were given that were essentially reviews rather than original scientific papers. In this way, practically the whole of the current field of polymer progress was summarized. The official languages of the Symposium were German, French and English, and during the morning sessions, translations of the text of the lectures were projected on to two screens behind the speaker, the third central screen being reserved for slides. On the whole, the system worked very well, provided the lecturer stuck to his text.

The afternoons were devoted to consideration of the numerous short communications; in each of the five main sections, about forty communications, which were intermediate in length and character between a paper and an abstract, had been submitted. Discussions of at least two, and sometimes three, sections were held simultaneously in the various halls, about twelve short papers, together with the morning lectures, being discussed at each session. While at some sessions the time allocated for discussion was more than adequate, at others the time for discussion of some papers was quite inadequate, and the chairman seemed more concerned to keep to his average 'driving speed' than to promote full discussion. This is a problem that besets all conferences, but perhaps the very size of this one made flexibility of arrangements more difficult. (It is only fair to say that numerous participants of the Symposium found no fault at all with the arrangements for discussions.)

Faced with more than thirty full lectures, and two hundred short communications, even the most indefatigable student of macromolecules feels surfeited, and becomes highly selective in his attendance. For this reason, and because at least two sessions were held simultaneously both in the morning and afternoon, I cannot give a full account of all aspects of the Symposium. The following summary covers what seemed the most interesting and significant features of the lectures and papers.

In the field of the physics of polymers, there was considerable interest and activity in studies of the mechanism of crystallization and the morphology of crystalline polymers. The lectures by A. Keller (of Bristol) and W. A. Kargin (of Moscow), in particular, aroused lively discussions. Kargin and also S. R. Usmanov (of Tashkent) presented the case that polymers that are normally regarded as partly crystalline, such as cellulose, are not truly crystalline in any sense, since they do not pass certain thermodynamical criteria, such as the presence of transition points, and should be regarded only as highly ordered. Keller reviewed current opinions on the structure of spherulites and the exciting recent developments in forming macro-crystals of polymers in which the polymer chains are folded back on themselves. The other main branches of physics on which attention was centred were dielectric and mechanical relaxation phenomena, nuclear magnetic resonance, and the thermal behaviour of polymers.

The study of the properties of solutions of polymers obviously still commands considerable attention, from both the theoretical and the practical points of view. P. Debye (of Ithaca) presented a new method of estimating molecular interactions from measurements of light scattering, and this gave hope that ultimately a new experimental tool for estimating intermolecular forces would develop. Rheological and viscosimetric studies, and in particular the deduction of molecular dimensions from these, comprised one section, while another dealt with the thermodynamics of polymer solutions, and the quantitative determination of parameters such as the virial coefficients, etc. Of considerable interest to experimentalists were a lecture by F. Patat (of Munich) reviewing osmotic pressure techniques and results, and papers by C. Kruissink (of Delft) on the theory, and H.-G. Elias (of Munich) on the practice, of using membranes partially permeable to the solute. Further refinements of conventional methods of measuring molecular weights and molecular weight distributions were described, but there did not seem to be any major new developments in this field.

In the section on elementary processes and kinetics of polyreactions, several lively sessions were held. One topic that naturally aroused much interest was ionic polymerization, with special reference to stereospecific polymerization. Valuable interpretative reviews were given by G. Natta (of Milan) on stereospecific polymerization, M. Szwarc (of Syracuse, U.S.A.) on anionic polymerization, and M. Magat (of Paris) on ionic polymerization initiated by ionizing radiation. There were also, of course, numerous short contributions on these topics. Ionic polymerizations are attracting considerable attention at present, as a result of the enormous commercial developments following the pioneer work of Ziegler and Natta, and it is certain that, despite the enormous work effort, much yet remains to be discovered. The present conference provided some evidence that development

has outpaced theory and interpretation, and once this is remedied, and the runners in the race get their second wind, more exciting progress will be made.

Fortunately 'steam-radio' (that is, free radical) vinyl polymerizations continue to be studied, with the work perhaps best described as being in the consolidation state. Valuable reviews of the applications to polymer studies of radioactive labelling and electron spin resonance were provided by J. C. Bevington and D. H. Whiffen, respectively (both of Birmingham). The industrially very important process of polymerization in heterogeneous media attracted some attention.

A session was held on the polymerization of caprolactam, at which all the papers were given by workers on the Continent.

One section was devoted to the discussion of natural polymers and model substances. W. Kuhn (of Basle) gave a fascinating lecture on the production of mechanical energy by macromolecular systems. Current interest in polypeptides was reflected by lectures by T. Wieland (of Frankfurt) on the organic chemistry of the formation of peptide links and C. H. Bamford (of Maidenhead) on synthetic polypeptides. In these two lectures, a fascinating contrast between the ingenuities of the organic and the physical chemist was displayed. Close as are these two studies to the processes of life itself, some studies that were reported on nucleic acids, cancer, and enzyme action were even closer, and demonstrated the extensive range of topics to which polymer science has a major contribution to make.

Starch and cellulosic chemistry was also treated in several contributions and lectures by E. Husemann (of Freiburg) and H. Sihtola (of Helsinki). One interesting new discovery by Ø. Ellefsen (of Oslo) was the preparation of a new crystalline modification of cellulose. Only a few papers were given on rubber, including a review by W. F. Watson (of Shawbury) of recent work on the mechanochemical reactions of polymers.

A very interesting section was that on what might be termed the preparative chemistry of organic and inorganic polymers. A review of new condensation polymers was given by H. Mark (of New York); this included an elegant demonstration of 'interfacial polycondensation', by means of which high-molecular weight polyamides are formed virtually instantaneously at room temperature. The happy term 'Instant Nylon' has been coined in the United States for this polymer. The application of classical organic reactions to vinyl polymers leads to many interesting new products, and progress in this field was summarized by G. J. Smets (of Louvain). Considerable interest is being currently shown in inorganic, and organic/inorganic polymers, and this was reflected in numerous short papers as well as set lectures by E. Thilo (of Berlin), H. Deuel (of Zurich), H. Krebs (of Bonn) and S. Nitzsche (of Burghausen). Various unusual organic polycondensation reactions, and methods of forming cross-linked polymers and block copolymers, also received attention.

It will be obvious from the above, highly inadequate, summary that there was scarcely an aspect of polymer science that did not receive attention. Whether this is the best programme for a conference is a matter for debate, as it can be argued that limitation of the subject-matter promotes a fuller and deeper discussion. There can be no doubt, however, that the Symposium once again fulfilled its main purpose of bringing together polymer scientists and promoting fertile interchanges of views.

Some of the main lectures have already appeared in *Die Makromolekulare Chemie*, and the remainder, together with the discussions, will appear in later issues.

L. Valentine

INDUSTRY AND THE TECHNICAL COLLEGES IN BRITAIN

An important paper on the relationship between industry and technical colleges was presented at a conference arranged by the Liverpool District of the Federation of British Industries at Chester on October 21. The speaker, Mr. L. S. Newton, of Pilkington Bros., Ltd., suggested that, despite the increased attention being paid by industry to the education and training of its personnel, there are three ways in which many firms are still not making a real contribution to the development of the educated man-power of Great Britain.

Many firms are still evading their responsibility for training young people and are content to recruit their craftsmen, technicians and technologists from other companies. Industry should accept the opportunity provided by the 'bulge' to analyse its requirements for the next ten to fifteen years to see how many young people it could now absorb to train for later years. Many firms also do not provide facilities for part-time release for study in the day-time—this applies not only to industry but also to professional and commercial undertakings. Industry frequently fails to discriminate between the branches of study, the facilities for commercial students remaining quite inadequate.

A feature of post-war development has been the remarkable rise in the number of students being released for sandwich courses. The number of students attending the technical colleges full-time or on a sandwich basis has risen tenfold in the past twenty years, between 80 and 90 thousand being in attendance at the moment. What the ultimate ratio should be has not yet become clear, but industry's support for sandwich courses is far from uniform and largely in the hands of the larger and more progressive companies.

Mr. Newton's most important contribution to the conference, however, was concerned with the ways in which closer co-operation between industry and the technical colleges could be achieved.

There are four main places where action could be taken to establish, maintain and foster relationships between the two bodies for their mutual benefit: (*a*) there should be a closer tie at the policy-making level; (*b*) there should be a closer tie at the point at which the contents of syllabuses and courses are determined; (*c*) there should be a closer tie at the teaching level; and (*d*) there should be a greater sharing of responsibility for the success of the student.

Probably the most important contribution that industry can make is to participate in policy formulation for the organization and administration of technical colleges by serving on college governing bodies and departmental advisory committees. Some colleges are still governed by a committee which is a sub-committee of the local further education committee, which itself is a sub-committee of the education committee of the local authority. "Moreover, we view with increasing concern the growing intrusion of local political issues into the deliberation of some governing bodies which has a discouraging effect on industrial participation."

Industry must co-operate in the teaching of technical colleges partly by encouraging more of its qualified young men and women to accept part-time teaching posts in the colleges and also by releasing, where necessary, men of appropriate skill and knowledge to teach on a part-time day basis. Sandwich courses in particular need teachers with close and current contact with industrial problems; these are not always to be found—nor could they be fully and efficiently employed in the technical college. Industry can offer another valuable service—that of taking in the full-time teacher for periods of refreshment in current practice. Local education authorities are empowered to release teachers for up to 12 months for industrial experience without loss of pension rights.

Since industrial firms supply the great majority of students, they must keep the college generally informed of their education requirements—qualitatively and quantitatively. They must keep the colleges informed of the latest developments in their specialized fields and of the needs to which the college can contribute. They must support the colleges in their activities and assist them in carrying out their job—whether by loaning staff or equipment or giving them the benefit of their knowledge in the advisory or administrative fields. They must consult with them on all matters of joint interest and maintain a constant flow of information in both directions.

The conference was attended by 140 representatives of education and industry from north-west England.

MODERN NETWORK THEORY IN ELECTRICAL ENGINEERING

THE first phase in the development of network theory was opened by the work of Ohm in 1827 and may be regarded as being terminated, at the end of the nineteenth century, by the work of Heaviside. By this date the main concepts and methods of network analysis were established and although mathematicians more orthodox than Heaviside were yet to present operational methods in a different light, little other work of significance appeared between 1900 and 1922. The subsequent growth of network theory was greatly stimulated by the use of the notion of a complex frequency, a concept which, in its turn, grew out of the application of the theory of functions of a complex variable to those problems of time-variation in networks which had previously been treated by the methods of Heaviside.

With the introduction of more powerful mathematical tools it became possible, in the second and present phase in the growth of network theory, to treat the problems of realizability, synthesis, approximation and practical design, that is to say, which mathematical functions may be realized as network characteristics? How may networks having prescribed, realizable, characteristics be found? By what approximations may a desired physical response be most usefully and economically translated into a set of realizable mathematical functions? In what manner do the general mathematical processes of realization and synthesis require to be treated if the outcome is to be a practicable network using a comparatively small number of relatively cheap components?

These are some of the questions with which modern network theory is concerned, and to discuss them the Department of Electrical Engineering of the University of Birmingham organized an informal conference which took place in that Department during September 21–24. The main purpose of the conference, believed to be the first on this subject to be held in Britain, was to give ample opportunities for the discussion of network problems, and the lectures and papers which were presented took up only one-half of the time of each session in order to permit this. In a limited space it is not possible to give equal treatment to every paper and discussion, but it is hoped that the nature of some of the problems discussed will be made clear by the following account.

Of all the sessions, the first was the most fundamental and abstract but also at the greatest remove from any immediate relevance to practical problems. Mr. P. R. Bryant (General Electric Co., Wembley) spoke on a topological analysis of the order of complexity of an electrical network. The 'order of complexity' was defined as the number of the natural frequencies of an electrical network and this number was shown to be equal to the number of dynamically independent network variables. In order to prevent too naïve an approach to this work it is only necessary to point out that a network of n capacitors connected in series possesses an 'order of complexity' of n, whereas the network formed by connecting the capacitors in parallel is of order 1. The other paper in this session was presented by Dr. V. Belevitch (C.E.C.E., Brussels) and was concerned with a treatment by matrix algebra of the realizability theory for general linear, stable networks with n terminal-pairs.

A discussion on a group of papers on networks with parameters varying with time was opened by the authors—Prof. D. G. Tucker, Mr. D. P. Howson and Mr. J. M. Layton (University of Birmingham). A major part of the discussion centred on the possibility of applying, to networks with parameters varying with time, theorems developed from a study of networks with constant parameters. That care is needed in making translations of this kind was shown by a demonstration in one of the papers that the input impedance of certain rectifier modulators was dependent on the nature and magnitude of the impedance associated with the source used to provide the input voltage and current. This result is more surprising than the discovery that theorems such as those of Thévenin and Norton cannot usefully be applied to many networks varying with time. Much more work is required to define the types of networks to which the main results of classical theory may be applied,

and it is evident that the analysis of networks containing reactances varying with time presents many problems, not the least of which is that of determining the conditions for stability.

In the third session Dr. O. P. D. Cutteridge (Manchester College of Science and Technology) spoke on the application of a particular continued-fraction expansion to a range of problems of active and passive networks. The expansion may also be used to determine the character of the zeros of a polynomial.

A lengthy discussion was initiated by a paper on the synthesis of three-terminal resistance-capacitance networks, presented by Mr. J. T. Allanson (University of Birmingham). There exists no complete solution to the problem of designing a network of this kind if the three impedance functions of the network are specified. It can be shown, by analysis of real networks, that the impedance matrix for any three-terminal resistance–capacitance network must necessarily satisfy certain conditions : it can also be shown, by synthesis of networks, that if an impedance matrix satisfies a more extensive set of conditions a network of this type may be found to represent the matrix. The paper and the discussion were concerned with attempts to close the gap between these two—the necessary and the sufficient—sets of conditions.

A discussion of a similar kind took place after Dr. W. Saraga (Siemens Edison Swan, London) had discussed the analysis and synthesis of reactance networks in terms of the 'one-points' of various functions. The traditional methods of analysis and synthesis have regarded the poles and zeros of various network functions as constituting the most easily handled specification of these functions, but there are certain advantages in using the values taken by the complex frequency variable when the functions in question have the values of $\pm$ 1. However, the simplicity of procedure which results from using these 'one-points' in network synthesis has to be paid for by an increase in the number of network elements required to realize a given set of functions. In practical applications these redundant elements must be removed and the series of network transformations necessary to do this may be sufficiently complicated to nullify the advantages of the preceding simplicity. It appears that 'one-point' analysis may be most usefully applied as complementary to pole-zero analysis in revealing useful general relations in networks.

Dr. G. S. Brayshaw (University of Leeds) presented a paper dealing with an application of positive-real graphs and Dr. A. Talbot (Imperial College, London) discussed the use of Tchebyscheffian approximations in network synthesis, and outlined an algebraic method for the determination of rational approximating functions suitable for filters required to have Tchebyscheffian characteristics in both pass and stop bands.

In the fourth session of the conference two papers were read on the design and construction of filter networks. Mr. E. R. Austin (Post Office Research Station, London) outlined a computational procedure for obtaining, in the form of a ladder network, an image parameter filter in which advantage is taken of the theoretical possibility of specifying independently the attenuation and impedance characteristics. The design may be regarded as yielding a filter more efficient than the corresponding Zobel type but less so than one designed on an insertion loss basis. Dr. J. H. Mole (H.M. Underwater Detection Establishment, Portland) spoke on problems of component design, accuracy and tolerance, mechanical construction and the testing and measurement of practical filters.

The final session of the conference was taken up with a discussion on the place and teaching of electrical network theory in universities and technical colleges. Mr. F. E. Rogers (The Polytechnic, London), Dr. A. W. Keen (Coventry Technical College), and Mr. E. R. Broad (Post Office Research Station, London) opened the discussion, which was vigorous but inconclusive. However, it was agreed that more attention must be paid to the physical and mathematical fundamentals underlying the practical techniques for the solution of network problems and less time given to these techniques themselves.

J. T. Allanson

BRITISH OVERSEAS INFORMATION SERVICES

In a written statement circulated in reply to a question in the House of Commons on November 16 as to the implementation of the proposals in the White Paper on Overseas Information, the Chancellor of the Duchy of Lancaster, Dr. C. Hill, stated that since April 1, 1959, the British Council has recruited 140 teachers of English for overseas posts, and expenditure on exchanges of university teachers with foreign countries has risen from £6,164 in 1958–59 to about £9,014 for 1959–60 ; for Commonwealth exchanges the corresponding figures are £12,113 and £16,113. The number of scholarships awarded by the Council has risen from 284 in 1958–59 to 355 in 1959–60, and of these 98 and 141, respectively, were related to the teaching of English, including twelve new scholarships for students from the Colonial territories for teaching English as a second language, 14 studentships at the University of London Institute of Education for United Kingdom graduates intending to teach English overseas, and fifteen new posts concerned with the teaching of English established overseas. Educational exchanges with Czechoslovakia and Hungary have commenced, including a three-week visit to British universities of six professors from Czechoslovakia. Government grants to the British Council were estimated at £5,824,000 for 1959–60, compared with £4,615,000 in 1958–59.

Expenditure on British Council libraries was £234,000 in 1958–59 and will rise to about £480,000 ; for books and periodicals for presentation, the corresponding figures are £39,000 and £72,000. In consultation with the Governments concerned, twenty new British Council libraries have been, or will shortly be, set up, and long-term lending library services for students are now operating or will shortly operate at nine Council centres and through universities. Libraries of the long-lending type are already operating in Karachi, Rawalpindi and Dacca and in process of being extended to Lahore and Chittagong, and first indications are that they are a great success. More than 30,000 books have been sent out and 235 additional subscriptions to periodicals provided for

Council libraries in India since April 1, 1959; for Pakistan, the corresponding figures are nearly 40,000 and 104. Measures to promote exports of British books and periodicals are making good progress, and proposals have been put to the Governments of Indonesia, Israel, Pakistan, Poland and Yugoslavia with the aim of agreeing upon schemes broadly on the lines of the former British book export schemes. Trade organizations in the periodical field have been consulted on measures to assist circulation of newspapers and periodicals in some of the more difficult markets overseas.

In broadcasting, Dr. Hill said that excellent progress has been made with the construction of the new relay transmitter at Berbera, and the Arabic Service of the B.B.C. is now transmitting for 12 hr. daily compared with 4·5 hr. in 1957. Efforts have been made to increase both the local and general appeal of the Service, and arrangements have been made to provide the local Press and broadcasting services in East Africa and Aden with more material on the background in the news. A conference of the Governments of the East African territories, Aden and the Somaliland Protectorate in March 1959 provided a valuable interchange of information and ideas, and the re-appraisal of the Overseas Service of the B.B.C. in English, nearing completion, has given full weight to the special importance of increasing the appeal of these Services to the rapidly growing number of listeners overseas for whom English is a second language. Studies have been made of the possibility of introducing television in Aden.

With regard to official services, a United Kingdom Information Office has been opened in Kampala and premises in Nairobi are being equipped; expenditure on television material for showing overseas is expected to be £138,000 in 1959–60 compared with £121,000 in 1958–59, and all the expansions recommended in the Central Office of Information Services have been made or are in progress. These include improvements in the Press and telegraph services, a radio-type service for local broadcasting in Arabic, Somali and Swahili; the completion of three experimental television films for the British Council on the teaching of English; increased supply of trade and technical periodicals overseas and an expanded programme of overseas visits, especially on the Commonwealth side. A Commonwealth Exhibition, intended as the focal point of 'Commonwealth Weeks' in several major cities, was opened in Liverpool on November 19. The cost of the Overseas Information Services in 1959–60 is now estimated at about £16·5 million a year, while measures to promote exports of British publications will cost about a further £500,000 a year.

METALLOGENIC MAPS

THE notion and the term metallogenic or metallogenetic are due to L. de Launay, who in 1900 proposed the terms 'metallogenic provinces' and 'metallogenic epochs'. Respectively, these two terms meant a particular geographical region or a specific geological epoch characterized by a particular assemblage of mineral deposits. The representation of such a province or provinces on the map led to the construction of metallogenic maps. Such a map differs from what is called a 'mineral map'. A mineral map only shows the distribution of one or several mineral deposits, while the aim of a metallogenic map is not only this but also to relate the distribution of such deposits to the geological formations or periods and to the tectonic features of the region. This leads to the discovery of particular areas of distribution or zones of occurrence; changes in the character of mineral deposits in time; association of certain chemical elements among the deposits, and many other features.

During the past few decades the problem of construction of metallogenic maps of various countries or on an international basis has been discussed. At the International Geological Congress held in Mexico City in 1956 a Sub-Commission for the Metallogenic Map of the World was created. During March–April 1958 this Sub-Committee, as a part of the wider Commission of the Geological Map of the World, met in Paris (*Nature*, **182**, 502; 1958). At this meeting a number of metallogenic maps of different countries were shown and discussed and the future programme outlined.

In the U.S.S.R. the problem of metallogenic provinces, epochs and maps were particularly discussed by the late Y. A. Bilibin (1901–52) and S. S. Smirnov (1895–1947), and at the present time this work occupies the forefront of geological research. Valuable contributions to the metallogeny of various ores were recently made by N. S. Shatsky, discussing the manganese ores (*Bull. Acad. Sci. U.S.S.R., Ser. Geol.*, **4**, 3; 1954), and phosphorite deposits ("Conf. Sed. Rocks, Acad. Sci. U.S.S.R.", **2**, 7; 1955). The metallogeny of Kazakhstan has been discussed by K. I. Satpaev (*Bull. Acad. Sci. U.S.S.R., Ser. Geol.*, **6**, 34; 1953) and A. I. Semenov (*Soviet Geology*, **58**, 110; 1957). The principles of regional metallogenic analysis have been discussed by V. I. Serpukhov (*Soviet Geology*, **43**, 27; 1955). The principles of metallogeny and the construction of metallogenic maps are discussed by E. T. Shatalov (*Bull. Acad. Sci. U.S.S.R., Ser. Geol.*, **9**, 37; 1958). An attempt at a general metallogenic survey of the territory of the Soviet Union and the construction of a metallogenic map of the U.S.S.R. was made by V. I. Smirnov (*Bull. Acad. Sci. U.S.S.R., Ser. Geol.*, **4**, 3; 1959 and *Priroda*, **4**, 17; 1959). Recently, E. A. Radkevich (*Bull. Acad. Sci. U.S.S.R., Ser. Geol.*, **5**, 125; 1959) has published a report of the All-Union Metallogenic Conference held in Alma-Ata during December 1958, at which 800 delegates and visitors were present and a number of papers read and discussed. A valuable summary of the work done in the Soviet Union in this field combined with a manual for the preparation of metallogenic maps has been published in book form. It is edited by P. M. Tatarinov, V. G. Grushevoy and G. S. Labazin, and it is entitled "General Principles of a Regional Metallogenic Analysis and of the Methods of Compilation of Metallogenic Maps of Folded Regions", published by "Gosgeoltekhizdat", Moscow, 1957 (150 pages; price 7r. 20k.). This volume contains seven articles dealing with the general principles of a regional metallogenic analysis, sedimentary and igneous formations, mineral deposits complexes, metallogenic zones and methods of compilation of metallogenic maps.

S. I. TOMKEIEFF

ULTRASONICALLY DISPERSED SODIUM

By M. W. T. PRATT and R. HELSBY

General Chemicals Division, Imperial Chemical Industries, Ltd., Widnes Laboratory, Widnes

MANY chemical reactions involving sodium benefit from the use of the metal in a highly dispersed form. The surface area available for reaction is then very large: for example, the surface area of spherical particles of sodium 1 micron in diameter is 6×10^4 sq. cm. per gm. Recently, we have prepared, by an ultrasonic technique, much finer dispersions of sodium in hydrocarbons than can be made by the high-speed stirring methods most commonly used.

The production of sodium dispersions ultrasonically is simple; a molten mixture of sodium and hydrocarbon dispersing medium is ultrasonically irradiated for a few minutes under an inert atmosphere (a patent has been applied for to cover the process). A laboratory apparatus capable of producing 200-gm. quantities of sodium dispersions by this method is sketched in Fig. 1. An ultrasonic magneto-strictive transducer with a resonant frequency of 25 kc./s. is sealed by a 'Neoprene' ring into a cylindrical 'Pyrex' reactor (15 cm. × 6 cm.). Ultrasonic irradiation is applied to a molten charge of sodium and dispersing medium at a power of approximately 250 W. for about 10 min., until the colour of the dispersion becomes constant, indicating that the equilibrium state of dispersion has been reached. The dispersion is then forced out of the reactor by pressure of inert gas into a container, from which the air has previously been displaced by inert gas.

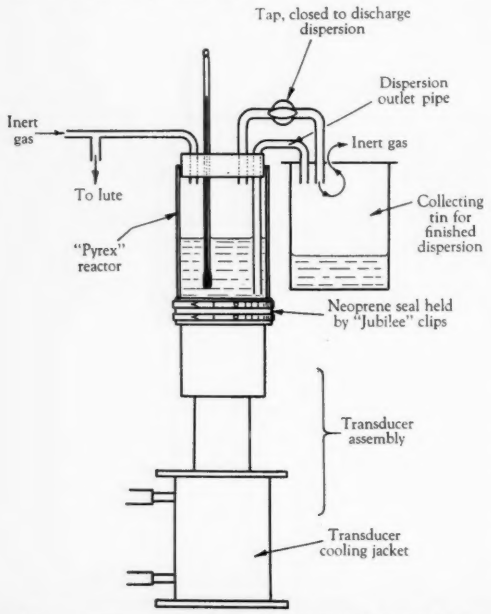

Fig. 1. Apparatus for the preparation of small batches of ultrasonically dispersed sodium

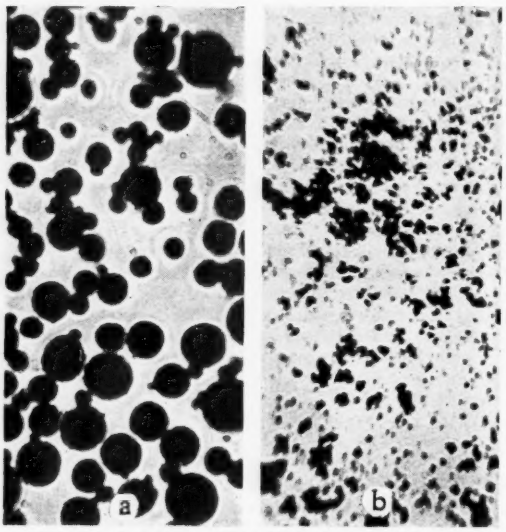

Fig. 2 (*a*). Mechanically dispersed sodium produced by stirring at 10,000 rev./min. (× 1,000)

Fig. 2 (*b*) Ultrasonically dispersed sodium; the small particles tend to agglomerate. (× 1,000)

Batch, or continuous-flow methods of dispersing sodium ultrasonically may be used; the latter enable quantities to be made on a commercial scale.

Any hydrocarbon with a boiling point higher than the melting point of sodium (97° C.) will serve as the dispersing medium, but the media we have found most useful are high-boiling (100–120° C.) petrol and yellow petroleum jelly. Dispersions can easily be made ultrasonically containing 15 per cent sodium by weight in petrol and 25 per cent by weight in petroleum jelly. Sodium particles in fluid hydrocarbons are rapidly attacked by air and water, and should be used immediately after preparation. Sodium dispersed in yellow petroleum jelly reaches an exceptionally fine state of subdivision, possibly due to the presence of a natural dispersing agent in the jelly. The sodium particles in this paste-like medium are protected from attack by air and moisture, so that the dispersions keep well, and can be safely transported. The sodium is liberated for reaction either by melting the jelly, or dissolving it in petrol; to avoid decrease in reactivity, this must be done under an inert atmosphere.

Stirring molten sodium and dispersing medium together mechanically at 10,000–20,000 rev./min. produces grey-coloured dispersions in which the particles are mainly between 3 and 15 microns in diameter. A photomicrograph of a typical mechanically produced dispersion in petroleum jelly is shown in Fig. 2*a*. Nearly all the particles in ultrasonically dispersed sodium in petroleum jelly are 1 micron or

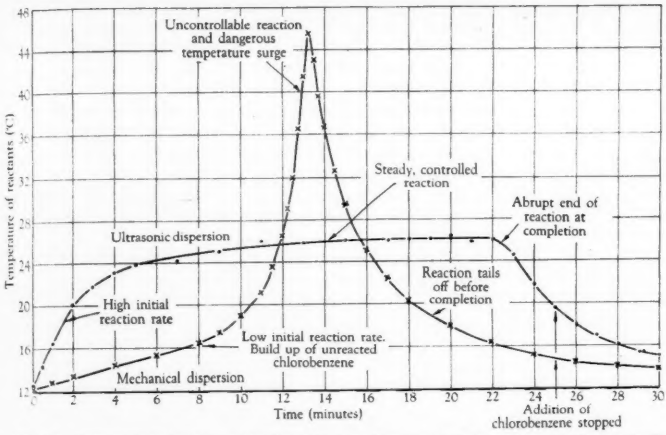
Uncontrollable reaction and dangerous temperature surge
Steady, controlled reaction
Abrupt end of reaction at completion
Ultrasonic dispersion
Reaction tails off before completion
High initial reaction rate
Low initial reaction rate. Build up of unreacted chlorobenzene
Mechanical dispersion
Temperature of reactants (°C)
Time (minutes)
Addition of chlorobenzene stopped

Fig. 3. Comparison of the reactivities of sodium dispersed mechanically and ultrasonically

less in size (Fig. 2*b*), and these dispersions have a deep blue or purple colour. This colour is probably due to the scattering of light by the small particles, analogously to the production of the blue colour of the sky by the light-scattering effect of dust particles.

Having established that ultrasonic techniques enable sodium to be dispersed to a higher degree than is possible by rapid stirring, we showed that the extra fineness produces advantages in chemical reactions greater than those gained by the use of mechanically dispersed sodium. This is well demonstrated in the exothermic reaction between sodium and chlorobenzene to produce sodium phenyl. This reaction is sometimes difficult to initiate, even when the best mechanically dispersed sodium is used. However, when sodium ultrasonically dispersed in petroleum jelly is used (afterwards dissolved for reaction in 100–120° C. petrol), reaction is always initiated at once, even at 20° C., which is a low temperature for this reaction. The reaction-rate is 10–20 times greater for ultrasonically dispersed sodium than for the mechanically dispersed metal.

In experiments performed with small quantities of reagents, chlorobenzene was added continuously, at the rate of 0·5 ml. per min., to 6 gm. of sodium dispersed in petroleum jelly, afterwards dissolved in 150 ml. petrol. Fig. 3 shows that, under these conditions, ultrasonically dispersed sodium immediately reacts at a high rate (indicated by the rapid rise in the temperature of the reactants towards its equilibrium value) and the reaction is smooth and controllable. Under the same conditions, mechanically dispersed sodium is not able to react quickly enough to prevent a build-up of unreacted chlorobenzene; eventually the accumulation of chlorobenzene reacts at an accelerating rate, and an uncontrollable temperature surge occurs. When this reaction has been carried out on a large scale, using mechanically dispersed sodium, temperature surges have sometimes been sufficient to boil the dispersing medium, and serious explosions have followed.

It is therefore advantageous to use ultrasonically dispersed rather than mechanically dispersed sodium in the preparation of sodium phenyl, and this is likely to be the case for many other reactions also. The benefits commonly claimed for the use of mechanically dispersed sodium instead of the bulk metal can now be extended in higher degree to the use of ultrasonically dispersed sodium, for which the advantages may be summarized as follow:

Faster reaction rates are obtained as a consequence of the large surface area of a fine dispersion.

Improved reaction control follows, for there is no accumulation of unreacted material, and the sodium can be added at an easily and precisely regulated rate.

Temperatures can be lower due to the high reactivity of the dispersions. Sudden temperature surges, which may occur with the use of bulk or mechanically dispersed sodium, are eliminated, and the reaction temperature may be held within close limits.

Elimination of side reactions frequently results from the use of low reaction-temperatures.

Higher yields result from the above advantages, and also because dispersions are completely consumed in conditions in which an insoluble reaction product coats the surface of bulk sodium, and prevents the reaction of a large fraction.

Replacement of more complex reagents such as sodium alkoxides, sodium hydride, and sodamide by sodium dispersions is possible in reactions which do not proceed satisfactorily with the bulk or mechanically dispersed metal.

Chemical reactions in which these advantages are likely to result include Claisen condensations, Wurtz reactions, preparation of sodium alkyls, aryls and alcoholates, metalations, replacement of active hydrogen atoms and the purification of hydrocarbons.

We are grateful to many colleagues in Imperial Chemical Industries, Ltd., who designed and built the ultrasonic equipment we used.

SWELLING FORCES IN STRAINING CLAYS

By E. L. CREASEN

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IT is generally accepted that the compression and swelling of a clay saturated with sodium ions is described by the osmotic theory of swelling[1–3]. This theory is based on osmotic effects associated with the adsorbed layer of ions in clays[4,5], and relates the thickness of the water layer between two clay plates to the swelling pressure[2]. In the case of calcium clays, however, although the virgin compression curve agrees reasonably well with the theoretical curve, the subsequent swelling on decompression does not. Warkentin, Bolt and Miller[3] concluded in this regard that either an unspecified attractive force was operating or that the osmotic swelling force was somehow diminished. If these conclusions can be

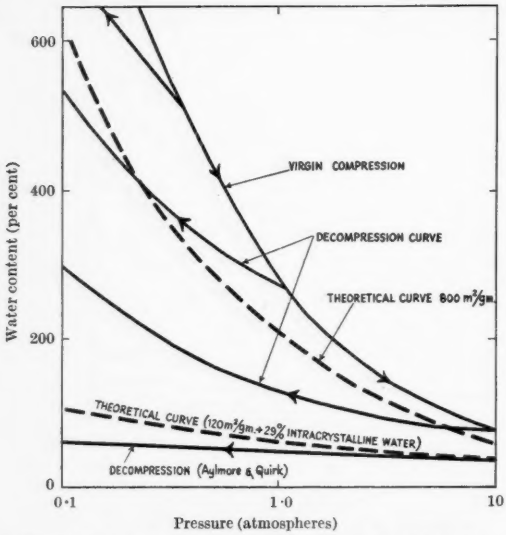

Fig. 1. Compression and decompression curves for calcium clay with theoretical swelling curves

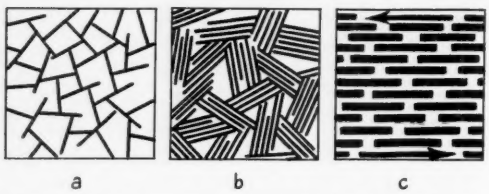

Fig. 2. Possible structure in (*a*) flocculated clay, (*b*) consolidated clay, (*c*) illite clay in the ideal straining *CVR* condition

accepted, they suggest an explanation of the behaviour of clays when they are strained under load.

Recently, however, Aylmore and Quirk[6] have questioned the interpretation of these results on the grounds that for the calculation of film thickness Warkentin *et al.* took as the specific surface of their calcium montmorillonite a value of 800 m.²/gm., whereas Aylmore and Quirk found that for a similar clay 648 m.²/gm. out of a total surface of 760 m.²/gm. was intracrystalline and not operative, giving an effective surface of only 112 m.²/gm. They concluded that the osmotic theory of swelling does not apply to clays saturated with calcium ions.

The point of difference can be explained readily by reference to Fig. 1. In order to overcome the above objection, I have plotted the results of Warkentin *et al.* as water content, in the place of film thickness, against the applied pressure. I have also included a probable swelling curve for Aylmore and Quirk's clay from the results they have given. The theoretical curves (broken lines) give calculated pressures for values of film thickness which would exist for the two cases where (i) the whole surface, 800 m.²/gm., is active and (ii) where approximately only one-seventh, 120 m.²/gm., is active and the curve is corrected for intra-crystalline water[6]. It appears that the calcium clay is becoming compressed from the virgin condition against a pressure that is somewhat higher than would be expected even from an osmotically active surface of 800 m.²/gm. I conclude that a separated plate model (Fig. 2*a*) is suggested by the results, and that this becomes compressed progressively to the condition shown in Fig. 2*b*, similar to that proposed by Aylmore and Quirk.

The interesting feature of the curves of Fig. 1 is the apparent relation between the theoretical swelling curve and the *CVR* line[8] of soil mechanics. When a virgin compressed clay is strained under shear, it becomes compressed further to a state where the voids ratio (water content) is in equilibrium with the compression load; this is called the critical voids ratio state and a plot of the critical voids ratio against the effective pressure is known as the *CVR* line. Furthermore, when an over-consolidated clay, that is, one that has been compressed and then unloaded to some point on the decompression curve (Fig. 2), is strained under a shear stress, it swells to an equilibrium voids ratio which is described by the same *CVR* line[7,8]. Consider now the model for the calcium clay during virgin compression. Edge to face bonds, or short-range attractive forces which could operate under the conditions of Fig. 2*a*, will assist the osmotic swelling force in resisting volume change during compression; but on the other hand they will oppose swelling forces during decompression. This explains why the theoretical curve falls below the virgin compression curve in Fig. 1. Further, if we accept the unique nature of the *CVR* line for a straining clay, it follows that short-range forces are not operating at all during shear, but that the *CVR* state represents a balance between only the osmotic swelling pressure and the external force. It is suggested that when a compressed clay (Fig. 2*a*, *b*) is strained under shear, the short-range forces are overcome, and the clay plates are fully oriented (Fig. 2*c*) at a spacing outside the range of these forces. I have described[9] a rheological model consisting of friction bodies and springs which allows for this explanation of the *CVR* state.

In Fig. 3 the *CVR* line[10] is given with compression and swelling curves[11] for a sample of London clay. This soil is illitic, with clay ($<2\mu$) content 69 per cent, specific surface 88 m.²/gm., and 15 m.equiv. of

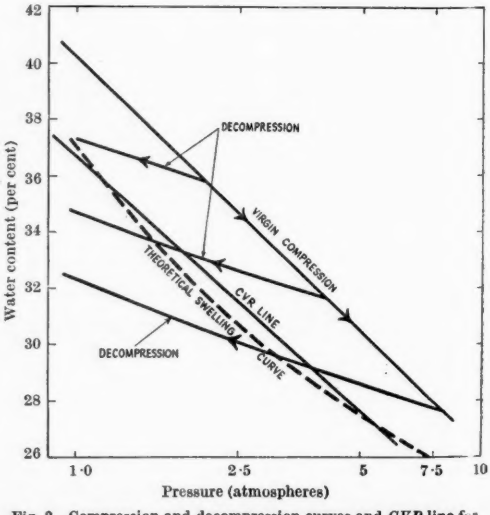

Fig. 3. Compression and decompression curves and *CVR* line for London clay. The calculated swelling curve for a solution concentration of 10^{-3} *M* has been arranged over the *CVR* line by a 'dead' volume correction of + 26 A.

exchangeable calcium out of a total exchange capacity of 19 m.equiv./100 gm. (ref. 12). From the above data the specific surface for the clay fraction is about 120 m.²/gm., which is the value for illite used by Bolt and Miller[2]. A value of 10^{-3} *M* has been assumed for the soil solution concentration, although this is not critical. On the assumption that the change in volume of the clay fraction accounts for the change in volume of the whole soil, I have calculated a theoretical swelling pressure curve. This curve has been arranged over the *CVR* line by correcting for a dead volume of water equivalent to a film thickness of 26 A. According to Bolt[1], this dead volume represents the volume of water which, due to the geometry of clay particles, cannot be expelled from the soil by compression. For London clay a correction for dead volume of 30 A. places the theoretical curve exactly over the virgin compression curve; for Weald clay, for which parallel data are available[10–12], a value of 36 A. is required. These agree with the 30–40 A. shift found by Bolt for a prepared illite. It should be noted that, although the position of the theoretical curve relative to the *CVR* line depends solely on the dead-volume correction, the two curves have the same slope. This shows that the osmotic theory can account for the change in volume of calcium illite under these conditions.

The above model also suggests a method for testing the existence and effect of long-range attractive forces in clays. Since such forces will oppose osmotic repulsion during both swelling and compression, their presence should not deny the unique nature of the *CVR* line as would short-range forces. On the other hand, since for a given external pressure the osmotic theory requires approximately double the interparticle spacing for a sodium compared to a calcium clay, one might expect the effect of long-range forces to differ for the two systems. Any such difference would at present be lost in the dead-volume correction.

The above results do not prove conclusively that the simple osmotic theory is applicable to calcium clays, but they do show that it can provide a reasonable working hypothesis in an otherwise obscure field. It should not be lightly discarded. The theory could be tested by measurements of the degree of orientation obtained during compression and compression with shear. Further progress seems to lie in a more critical estimation of the *CVR* concept and of the dead-volume factor for both sodium and calcium clays in the straining condition.

Acknowledgment for helpful discussion is due to Drs. W. W. Emerson and K. Norrish and Mr. J. W. Holmes, all of the Division of Soils of this Organization.

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A SPECIFIC RESPONSE IN THE HIPPOCAMPUS OF THE RABBIT TO OLFACTORY STIMULATION

By B. G. CRAGG*
Department of Anatomy, University College, London

THE olfactory bulb projects to the piriform lobe through the lateral tract and through the more medial radiation which is continuous with the anterior limb of the anterior commissure. The distribution of these tracts within the piriform lobe has been described in experimental material stained by the method of Glees[1,2]. Terminations are seen mainly in the prepiriform cortex, anterior olfactory nucleus, olfactory tubercule and central amygdaloid nucleus. The projection to the last-named area is probably derived from the medial radiation rather than from the lateral tract, and is bilateral. No terminations have been seen in the hippocampus or in the septum. The distribution in the piriform lobe of electrical responses to olfactory stimulation is substantially compatible with the histological findings[3–5]. An electrical response in the hippocampus to single electrical stimuli applied to the olfactory bulb has however been claimed[6] but not confirmed. The purpose of this article is to describe a large and consistent electrical response in the hippocampus of the rabbit that can be obtained by suitable repetitive stimulation of the olfactory tracts.

Electrical recordings were made from an insulated needle with a bare tip of diameter 10μ placed in the pyramidal cell layer (Ammon's horn) of the hippocampus while electrical stimuli were applied to a radial pair of needles placed in the olfactory peduncle to stimulate both the medial radiation and the lateral tract. Stimulation at a frequency less than 8 per sec. sometimes elicited very small evoked responses in the hippocampus. At a frequency of stimulation between 8 per sec. and 20 per sec., however, large evoked responses invariably occurred throughout the dorso-ventral extent of the ipsilateral hippocampus, but only after 50–100 stimuli had been delivered. At frequencies between 20 per sec. and 230 per sec. no hippocampal response was seen, but if the stimulation rate were switched down to 5–20 per sec. after 50–100 stimuli had been delivered at a higher frequency, large evoked hippocampal responses were obtained after 5–10 stimuli had been given at the slower rate.

Evoked hippocampal responses were obtained by stimulation of the medial radiation alone, but not by stimulation of the lateral tract alone. If, however, repetitive stimulation were suddenly switched from electrodes in the medial radiation to electrodes in the

* Leverhulme Mental Health Research Fund Fellow.

lateral tract while the hippocampus was responding, the responses continued unchanged except for a small increase in latency. The same result was obtained on switching from the ipsilateral to the contralateral medial radiation, for hippocampal responses can be continued but seldom started by contralateral stimulation. A similar switching of repetitive stimulation from the medial radiation to the central cut end of the optic, sciatic or vagus nerves immediately terminated the evoked hippocampal responses. Moreover, if repetitive stimulation were applied both to one of these nerves and to the medial radiation until hippocampal responses were obtained, the evoked responses ceased at once if the olfactory stimulation were stopped while the nerve stimulation was continued. These hippocampal responses in the rabbit are therefore specifically related to the olfactory system.

The hippocampal response first appears after 50–100 stimuli as a small deflexion with a latency of 40–60 msec.; but with 5–10 more stimuli the latency decreases to 20–24 msec. and the full amplitude of up to 3 mV. is attained. With repetitive stimulation at 8 per sec. this response persists for 30–40 sec. after which the amplitude declines and the latency increases until the response disappears again with a latency of 60 msec. Apart from rare immediate recurrences, no further response can then be obtained until 90 sec. have elapsed without stimulation, although the response can be recalled for a few seconds by an early high-frequency tetanus. If the repetitive stimulation is stopped when the hippocampal response has just attained the full amplitude there is a brief after-discharge.

Electrical recordings have been made at different depths in the hippocampus to identify the elements responding (Fig. 1). Negative wave-forms are seen at the level of the pyramidal neurones in area CA_1 but positive wave-forms appear at the level of the dendrites in the upper blade of the dentate gyrus, and persist in area CA_4 and in the rest of the dentate gyrus. Recordings on other electrode tracks show a negative response in area CA_2 but not in area CA_3.

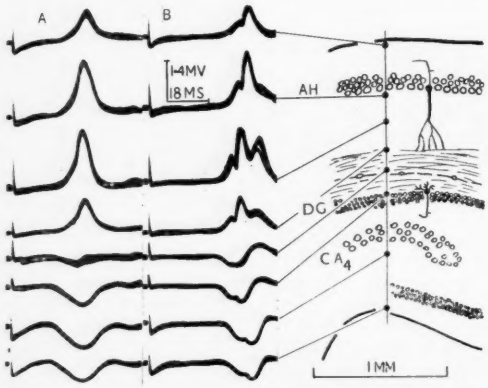

Fig. 1. Electrical recordings made at different depths in the dorsal hippocampus of a rabbit anæsthetized with urethane during the stimulation of the ipsilateral olfactory peduncle at 8 per sec. The diagram shows the positions of recording on a tracing of a frontal section through the needle track. A single neurone has been represented in the Ammon's horn with the apical dendrite below the cell body, and the axon above. In the dentate gyrus another single neurone is represented with the dendrites above and the axon below the cell body. *A*, the wave-forms recorded after 10 sec. of stimulation; *B*, after a further 30 sec. of stimulation, negative polarity upwards; *AH*, area CA_1 of the Ammon's horn; *DG*, upper blade of the dentate gyrus

The interpretation of these findings is that only the pyramidal cells of areas CA_1 and CA_2 respond to the stimulation. The same distribution of potentials is seen throughout the dorso-ventral extent of the hippocampus.

This hippocampal activity might be expected[7] to lead to visceral changes. Recordings of visceral activity show that the blood pressure rises slightly and the respiration is often increased in the period between the beginning of olfactory stimulation and the occurrence of the hippocampal response. When the latter appears the blood pressure starts to fall, the respiration is reduced, and the bladder and rectum contract. These latter effects soon pass off, but the fall of blood pressure continues as long as the hippocampus continues to respond, and the total fall may exceed 40 mm. mercury. When after 30–40 sec. the hippocampal responses cease, the blood pressure promptly rises again although the stimulation is continued. The low level of blood pressure is not the cause of the eventual failure of the hippocampal response, for the latter may occur at 80 mm. mercury after a fall from 120 mm. mercury, whereas a hippocampal response can be started with olfactory stimulation at a blood pressure of 40 mm. mercury though not at 25 mm. mercury.

These visceral changes coincide with, but are not in fact caused by, the activity of the hippocampus, for the fornix can be cut on both sides and the hippocampus dissected out with a sucker without affecting the visceral changes. It was found that the electrical responses of the hippocampus were unaffected by cutting the fornix or the fimbria, but were abolished by section of the angular tract of Cajal[8] at a position ventral to the point of recording. This finding, together with the fact that the shortest latency of the hippocampal response is 2–3 msec. less in the ventral than in the dorsal hippocampus, implies that impulses reach the dorsal hippocampus by ascending the angular tract from the piriform lobe. Activity of neurones in the latter concurrent with the hippocampal response is believed to cause the visceral changes. Similar visceral effects can be elicited by direct stimulation in the prepiriform cortex after ablation of the hippocampus. Impulses are presumably relayed from the prepiriform cortex to autonomic centres in the hypothalamus or brain stem.

Electrical recordings have been made throughout the piriform lobe during olfactory stimulation in order to trace the olfactory impulses to the hippocampus. Responses to single shock stimulation of the lateral tract were found ipsilaterally in the olfactory tubercule and throughout the prepiriform cortex as far caudally as the periamygdaloid cortex. The responses had a latency of 2–5 msec. to first inflexion and reversed sharply in polarity, being positive dorsal to the cell bodies and negative ventrally in the region of the axo-dendritic synapses. Similar responses and some later deflexions were obtained in these same areas, and also bilaterally in the amygdaloid complex, with single shocks applied to the medial radiation in the olfactory bulb after cutting the visible part of the lateral tract.

When repetitive stimulation is applied to the medial radiation the primary responses described above continue; large secondary responses appear throughout the piriform lobe but not in the posterior superior entorhinal cortex at the same instant that the hippocampal response appears. These secondary responses vary in latency from 6 msec. in the olfactory tubercle to 18 msec. in the amygdala. At any

position of recording the latency gradually increases after 10–20 sec. of stimulation. In the amygdala and in the caudal prepiriform cortex the secondary responses eventually disappear at a latency of 50–60 msec. at the same time that the hippocampal response disappears. In the olfactory tubercule and in the more cranial prepiriform cortex, however, the secondary responses increase in latency to about 20 msec., but may persist indefinitely with continued stimulation. The secondary responses are negative in polarity dorsal to the pyramidal cell bodies (where the primary response is positive), and reduced in amplitude but still mainly negative in polarity ventrally where the primary response is negative.

After recordings had been made at each placement, stimulation was applied to the needle electrode in the prepiriform cortex and further recordings made from the hippocampus. It was found that a large hippocampal response can often be elicited by single shock stimulation of the prepiriform cortex at intervals of not less than 1–2 min. With more rapid repetitive stimulation a hippocampal response was obtained only after many stimuli had been applied, the number increasing at more cranial placements of the stimulating electrode. The hippocampal response to stimulation at the more cranial placements gradually increased in latency and eventually disappeared, but stimulation in the more caudal prepiriform cortex elicited earlier hippocampal responses which persisted indefinitely with continued stimulation.

The primary responses in the prepiriform cortex to single shock stimulation of the lateral tract or the medial radiation were unchanged when the stimulation was made repetitive until the secondary responses appeared in the piriform lobe and hippocampus. At this instant the primary response was slightly reduced, and with continued stimulation the primary response progressively decreased to about a third of the initial amplitude at cranial positions of recording, or about a tenth of the initial amplitude in the periamygdaloid cortex. When the hippocampal response disappeared the primary response partially recovered, but complete recovery never occurred during continued stimulation. Complete recovery required at least 40 sec. without stimulation. This inhibition of the primary response took place without apparent change of latency and was not due to hippocampal activity, since it was unaffected by section of the fornix and angular tract.

The inhibition of the primary response increases at more caudal positions of recording, and so does the amount by which the latency of the secondary response can increase. These results suggest that the olfactory impulses are relayed through many neurones in the prepiriform cortex towards the ventral hippocampus, and that both excitatory and inhibitory connexions are activated. It was found that when small lesions were made in the prepiriform cortex, degenerating axons could afterwards be traced caudally by the Nauta-Gygax technique. Many of these axons end in the prepiriform cortex, but others continue in layer 1 through the entorhinal cortex to the subiculum of the ventral hippocampus. It will be of interest to see whether the remarkable changes of latency that occur in transmission through the prepiriform cortex are common to other systems in which multiple short and long connexions are made. A specific relationship of the hippocampus to the olfactory system is unexpected[9,10], for the spontaneous electrical rhythms of the hippocampus can be changed by afferent impulses of any modality[11]. The specificity of the large hippocampal responses in the rabbit to olfactory stimulation may be a quantitative effect due to the relative numbers of afferent impulses reaching the hippocampus from different sources, and must be tested in other species of mammals.

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ACCUMULATION OF RADIOACTIVE IODINE IN THYROID GLANDS SUBSEQUENT TO NUCLEAR WEAPON TESTS AND THE ACCIDENT AT WINDSCALE

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THE changes in thyroidal content of iodine-131 in grazing animals due to nuclear weapon tests have been shown to be a sensitive method of detecting fall-out of iodine-131 (refs. 1–5). From their observations that a similar concentration of iodine-131 was found in all thyroid glands tested, irrespective of whether they were from carnivores or from herbivores, Gunther and Jones[3] tentatively concluded that the iodine-131 had been absorbed by these animals through the lungs, having been inhaled as an aerosol. Doubt was shed on this suggestion by the results of Van Middlesworth[2], who showed that the concentration of iodine-131 in human thyroids was never more than 0·5 per cent of the maximum observed in cattle. This suggested, in so far as the grazing animal is concerned, that the alimentary tract may be the main route of entry of iodine-131. This was confirmed by Marston[5], who found very little or no iodine-131 in the thyroid gland of cats, dogs and rats at a time when the concentration of iodine-131 in

herbivora grazing on pasture in the same region was high. Stall-fed sheep had an activity of less than 0·1 per cent of the activity of grazing sheep.

In this report, values for the iodine-131 content of the thyroids of sheep grazing in north-east Scotland are given. These values cover the period November 1957–July 1959. Values for deer thyroid glands from north-east and from north-west Scotland and for human thyroids from the London area have been obtained during the period December 1958–March 1959. Shortly after the accidental release of iodine-131 from Windscale No. 1 pile on October 10, 1957, sheep thyroid glands were obtained from the Cumberland area during the period October 1957–March 1958.

All the measurements of activity reported were made using a commercial scintillation detector fitted with a 2½-in. well-type crystal. By the addition of an extra 2 in. of lead to the top and sides of the detector, the background radiation count was reduced to approximately 500 counts per 100 sec. When the detector was calibrated with a standard iodine-131 solution, it was found that $5{\cdot}0 \times 10^{-12}$ c. gave a count-rate sufficiently high to be statistically greater than background, at 95 per cent probability. All estimates made were determined by measuring the time taken for 10,000 counts ; hence the standard deviation of all counts will be constant.

An initial sample of sheep thyroid glands was collected at local abattoirs from sheep of known origin, trimmed free of connective tissue, weighed, homogenized and the count-rate determined on both lobes separately. These thyroids were recounted at weekly intervals over a period of a month. The half-life of the gamma-activity was found to be 8 0 ± 0·1 days and the decay curve, plotting the logarithm of activity against time, was linear and parallel to that of pure iodine-131. It was found that the presence of muscle tissue did not significantly affect the counter calibration with reference to iodine-131, so that the thyroid content of iodine-131 could be accurately expressed in absolute units, that is, curies. All results are expressed in terms of iodine-131 content per gram of fresh weight of thyroid tissue. All values are corrected to time of slaughter. A statistical analysis of the iodine-131 content of the right and of the left lobe revealed that no difference existed between them ($P = 95$ per cent). Afterwards, only one lobe was collected from each animal sampled.

In order to compare the effect of locality within the general region of north-east Scotland, groups of approximately ten lobes were collected from three batches of sheep from different areas slaughtered on the same day and the iodine-131 content determined.

The results were :

Fyvie	$2{\cdot}91 \pm 0{\cdot}28 \times 10^{-10}$ c./gm.	(8 animals)
Oldmeldrum	$2{\cdot}14 \pm 0{\cdot}12 \times 10^{-10}$ c./gm.	(10 animals)
Banff	$3{\cdot}34 \pm 0{\cdot}84 \times 10^{-10}$ c./gm.	(10 animals)
Overall mean	$2{\cdot}79 \pm 0{\cdot}32 \times 10^{-10}$ c./gm.	(28 animals)

There is no significant difference between these groups ($0{\cdot}2 < P < 0{\cdot}3$).

It should be noted that these localities lie more on the coastal belt of north-east Scotland rather than in the more mountainous hinterland. This general area, from which the majority of the values to be reported were obtained, has a fairly uniformly distributed rainfall of approximately 30 in. a year. The main advantage in drawing samples from this area is that it is possible to obtain these samples at regular intervals throughout the year. This is not the case with animals from the more mountainous areas with higher rainfall. The majority of the flocks sampled were from localities at least ten miles distant from the sea. In this region the prevailing wind is south-westerly and therefore off-shore. The possibility of sea spray depositing non-radioactive iodine on the pasture during in-shore winds is not considered to have materially affected the results obtained.

At certain times of the year it was only possible to obtain the thyroid lobes from fat lambs. It was therefore necessary to carry out a comparison between the activity of ewe and lamb thyroid glands. The results for one such comparison is given below :

Ewe thyroid activity	$4{\cdot}21 \pm 0{\cdot}35 \times 10^{-11}$ c./gm.	(16 animals)
Lamb thyroid activity	$7{\cdot}14 \pm 0{\cdot}64 \times 10^{-11}$ c./gm.	(8 animals)
Overall mean	$5{\cdot}2 \pm 0{\cdot}42 \times 10^{-11}$ c./gm.	

The mean for the activity of the lamb thyroids is significantly higher than that for the ewe ($P < 0{\cdot}01$). From other experiments carried out in this laboratory on the thyroid activity in ewes and lambs, it would appear that the difference between the iodine-131 content of their thyroids will vary according to whether the measurement is made during a period of accumulation due to recent fall-out of iodine-131 or during a period when the iodine-131 in the pasture is decreasing. The lambs, having more active thyroids than the mature ewes, would accumulate fall-out more rapidly than older animals and also excrete iodine more rapidly. The maximum difference is likely to be relatively small, considering that the overall magnitude of change encountered is of the order of one thousand times.

In order to obtain some information on what proportion of the thyroid content of iodine-131 could be attributed to absorption through the lungs and ingestion from drinking water, 16 wethers were randomly selected from a flock during a period of relatively low activity, namely, January 1958. Of these animals a group of 8 was brought indoors, where they were kept until slaughter in May 1958, that is, some five months later. During this period they were fed on hay and oats harvested during the previous season and kept under cover to preclude contamination by direct fall-out. The remaining 8 animals were allowed to run on normal pasture until slaughter. Both groups of animals were killed in May 1958 at a time when thyroidal activity due to fall-out in the groups of sheep was known to be high. The activity found in the thyroid glands of these two groups was :

Stall-fed animals	$1{\cdot}56 \pm 2{\cdot}2 \times 10^{-11}$ c./gm.
Grazing animals	$2{\cdot}25 \pm 0{\cdot}13 \times 10^{-9}$ c./gm.

The values found for the stall-fed animals are significantly higher than the minimal detectable level and higher than some values obtained from sheep on free grazing during other periods, suggesting that the stall-fed animals had been exposed to iodine-131 by inhalation and from drinking water. The hundredfold difference in these values suggests that in so far as the sheep is concerned, the main source of thyroidal iodine-131 is contaminated pasture, something of the order of 0·5 per cent arising from inhalation and drinking water.

Having established the validity of the counting and sampling techniques, arrangements were made to carry out regular determinations at approximately fourteen-day intervals. The values obtained are presented in Fig. 1, which includes histograms indicating the frequency and number of atomic weapon tests and the distribution of rainfall.

From the graph it can be seen that the minimal detectable level of 5×10^{-12} c./gm. is reached 2–3

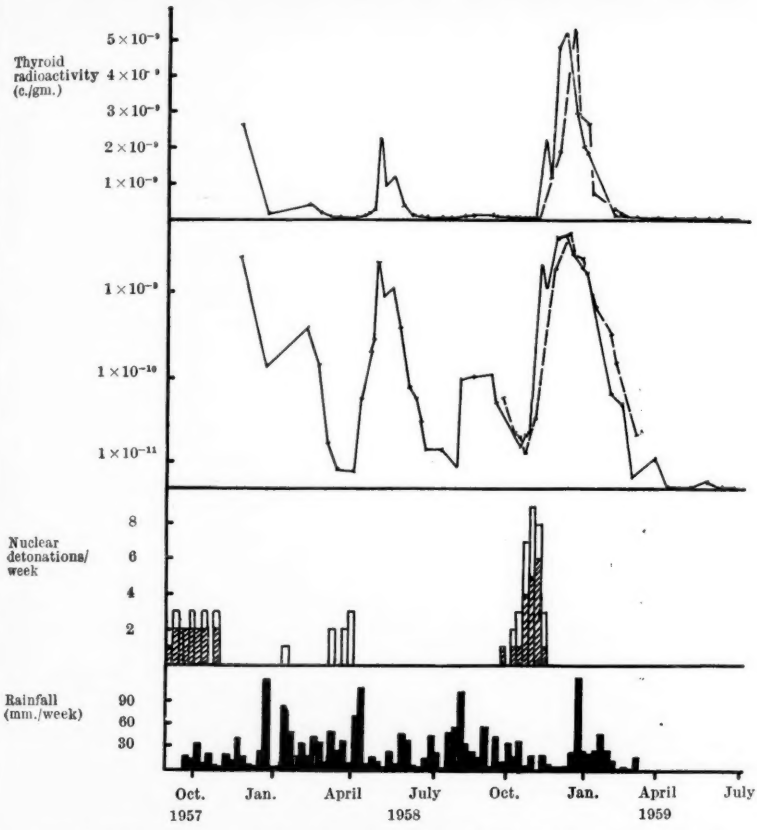

Fig. 1. Variation in thyroid iodine-131 content of sheep in the north-east of Scotland during November 1957–July 1959 with histograms indicating the frequency of nuclear detonations in the northern hemisphere and the rainfall. Nuclear detonations are indicated as: shaded (United States tests), unshaded (U.S.S.R. tests)
Superimposed on the upper graphs between September 1958 and February 1959 is the iodine-131 content of deer thyroids collected in north-western Scotland (broken line)

months after a test series. This is in keeping with a half-life of approximately 7 days for iodine-131 in the thyroid of sheep. This value of 7 days takes account of both the biological and the physical half-life of iodine-131 in the thyroid.

It has been supposed that only tests carried out in the northern hemisphere would influence iodine-131 levels in this area. However, it is not possible to relate the increased level found between July and September 1958 with any known northern hemisphere test immediately prior to this period. The Russian series of tests which commenced on February 23 ended on March 21 and did not commence again until September 30. All the American and British tests during the period April 28–August 12 were carried out in the southern hemisphere. It would appear, therefore, that either some unknown release of iodine-131 into the atmosphere took place in the northern hemisphere during this time, or that the debris from tests in the southern hemisphere had been detected in this area.

Towards the end of 1958 arrangements were made to obtain deer thyroids from both north-east and north-west Scotland. The values obtained from these two areas agreed very closely, but since more regular samples were obtained from the north-west, only the values for this area are shown (Fig. 1). Although the variability and standard error are higher for the deer measurements, due in most cases to small numbers in the samples, it would appear that the pattern for sheep and for deer is very similar.

During the period December 1958–January 1959, human thyroid glands obtained at post-mortem in the London area had a mean value of $1{\cdot}45 \pm 0{\cdot}37 \times 10^{-11}$ c./gm. (10 individuals), which is approximately 1 per cent of the value found in the lobes of sheep during this period. Since this occurred at a season of the year when fresh vegetables would be scarce, and milking cows would be fed mainly on stored fodder, it is suggestive, when compared with the stall-fed sheep experiment, that inhalation of iodine-131 by the lungs together with iodine-131 absorbed from drinking water represented in the adult human the major route of entry of iodine-131 during this period. The number of human thyroids sampled at any particular period was too small to obtain accurate information regarding how the iodine-131 content of the human thyroids was changing with time in relation to the iodine-131 content of the sheep thyroids.

Calculations of radiation dosage to the thyroids due to both β- and γ-emissions from iodine-131 have been made. These are based on a calculated radiation dosage of 11·2 rads per day per 1 μc. In the Aberdeen area during January 1–December 31, 1958, the calculated radiation dose to the thyroid glands of local sheep was 3·0 rads. During the period December 15, 1958–January 15, 1959, which is approximately six weeks after a series of twenty-nine atomic and hydrogen explosions in the northern hemisphere, the thyroid radiation dose due to iodine-131 has been calculated for sheep from north-east Scotland, deer from north-west Scotland and for humans from the London area. These values are:

Sheep	0·32 rad
Deer	0·31 rad
Human	$5{\cdot}9 \times 10^{-3}$ rad

It must be emphasized that since the number of human thyroids sampled was small, the value calculated for the human cannot be taken as more than an approximation.

Sheep thyroid glands were collected in the Windscale area following the reactor incident on October 10, 1957. During the period October 26, 1957–March 13,

1958, thyroid glands were obtained from 77 sheep. The first sample was from sheep killed on October 26, by which time the shorter lived isotopes of iodine would have decayed away. Half-life determinations were done on the gamma-activity of the thyroids, the half-life being 8·1 days and the logarithmic decay curve linear with time. A fairly wide scatter of values was obtained due to the sheep sampled coming from farms in different directions and distances from the Windscale pile.

The highest values obtained were from four sheep killed on November 10, that is, 31 days after the release. These animals had been obtained from two neighbouring farms situated 3,000 and 5,000 yd. respectively south-south-east of Windscale. This was within the area of maximum deposition[5]. Their thyroids had a value of $2{\cdot}0 \pm 0{\cdot}03 \times 10^{-6}$ c. iodine-131 per gm. Further samples were obtained from this locality on November 27, $1{\cdot}5 \times 10^{-7}$ c. per gm. (2 animals), and on March 13, 1958, when a value of $2{\cdot}2 \pm 0{\cdot}43 \times 10^{-11}$ c. per gm. (14 animals) was obtained. These three values gave a good fit to an exponential curve of half-life 7·3 days. This compares with a mean half-life of 7·0 days (18 animals) obtained experimentally in this laboratory.

An attempt has been made to obtain an approximate value for the radiation dose received by the thyroids of the sheep in the above locality. Experiments carried out in this laboratory have shown that, following the administration of a single dose of iodine-131 to sheep, the maximum activity in their glands is attained at a mean of 3·7 days (20 animals). A value of four days has therefore been taken as the shortest time to reach maximum activity in the thyroids of the Windscale sheep. This of course erroneously assumes that all the activity is washed off the pasture by rain after the first day. Under ideal conditions, that is, when no activity is lost from the pasture other than by physical decay, the accumulation of iodine-131 in an animal would follow the equation:

$$X = X_0 t \exp(-t)$$

where X is iodine-131 in the thyroid at time t; X_0 is iodine-131 originally deposited on the amount of fodder eaten in a day by an animal; decay constant of iodine-131, 0·0866.

This equation gives a maximum value when $t = 12$ days. A value of 12 days has therefore been taken as the longest time required to reach maximum activity in the thyroids of the Windscale sheep. An exponential curve of half-life 7·3 days was drawn through the observed points and the radiation dose to the thyroid calculated over a period of three months following the release. Assuming the time of peak activity of the glands to be four days after release, the radiation dose to the thyroid gland is approximately 4,500 rads, and assuming a peak activity at 12 days the dose is approximately 2,800 rads. It is thought that a value between 3,000–4,500 rads represents a fair estimate of the radiation dose received by the thyroids of these animals. It can be calculated from the activity of the sheep thyroids in the Aberdeen area over this three-month period that only 1·5 rads can be attributed to fall-out.

Little information is available regarding the radiation dose required to produce abnormalities in sheep thyroid glands. The experiments of Bustad *et al.*[7] suggest that changes in thyroid activity occur following the administration of a radiation dose of 5,000 rads over a period of six months.

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FORMATION OF DIFFERENT CELL–VIRUS RELATIONSHIPS IN TUMOUR CELLS INDUCED BY POLYOMA

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THE polyoma virus can induce tumours in mice, hamsters, rats and rabbits[1–4]. With the development of a plaque assay for this virus[5], it has become possible to undertake quantitative studies on cell–virus relationships and to produce genetically pure lines of virus. The use of such lines has shown that single-plaque isolates can induce multiple tumours in different species (ref. 5 and unpublished work). An experimental system is thus available for the study of a variety of mammalian tumours induced by the same virus, and the present article is concerned with experiments that demonstrate the existence of strikingly different cell–virus relationships in different tumours induced by polyoma.

The plaque assay, and hæmagglutination, were carried out as previously reported[5,6]. The growth medium for cell cultures consisted of 0·5 per cent lactalbumin hydrolysate in Earle's saline and 20 per cent horse serum.

In the first experiment, release of virus from seven mouse tumours induced in six mice (4 parotid tumours, 2 mammary adenomas and 1 kidney sarcoma) was determined by placing the cells in culture and testing the hæmagglutination titres of the culture fluid at various intervals. For each tumour, 5×10^6 tumour cells dispersed in trypsin were plated in 50-mm. Petri dishes with 4 ml. of culture medium, and the entire medium was changed,

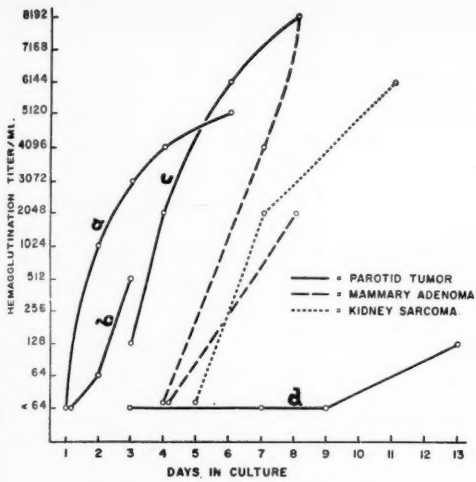

Fig. 1. Release of virus from mouse tumour cells in culture. The results are expressed as cumulative hæmagglutination titres per ml. of tissue culture fluid; (*a*) *MP* 2; (*b*) *MP* 6; (*c*) *MP* 3; (*d*) *MP* 11

and tested for hæmagglutination titre, on the days shown as points on the curves (Fig. 1) The titre obtained on a particular day has been added to the total of the previous titres, and the results are expressed as cumulative titres per millilitre of culture fluid. Titres less than 64 per ml. were not added to the cumulative results, since these were equivalent to the control value found for normal tissue culture fluid.

The results (Fig. 1) show that the release of virus in these cultures varies considerably even in different tumours of the same type. The histology of the tumours and the appearance of the cells in culture make it unlikely that these differences in release of virus are merely due to possible differences in the proportion of normal cells in the various tumours.

The results in Fig. 1 also indicate that in all the mouse tumours tested there was a 'lag' period before high hæmagglutination titres were obtained from the cells in culture, and that the length of this 'lag' period varied in different tumours. Since extracts of mouse tumours contain heat-labile inhibitors and high antibody titres[6], tests were made for the presence of inhibitors and antibodies during the 'lag' period. These tests all gave negative results, so that differences in the 'lag' period may be due to difference in rates of synthesis of virus and/or release of virus.

In order to determine the proportion of cells that release virus, cells from three parotid tumours, *MP* 10, *MP* 11 and *MP* 4, were plated on mouse embryo monolayers (*MP* 4 when plated alone gave hæmagglutination titres of 1,024–2,048 per ml. a day, from the second day in culture; and *MP* 10 a titre of 512 ml. during a three-day period from the second to the fifth day in culture). The proportion of cells releasing virus was determined by the relationship between the number of tumour cells plated and the number of plaques. The results (Table 1) show that one of the factors in the difference in release of virus from cultures of these tumours can be a difference in the proportion of cells that release virus.

Mouse tumours can thus release variable but considerable amounts of virus. In a total of sixteen

Table 1. Proportion of Cells releasing Virus in Mouse Parotid Tumours

Tumour	No. of tumour cells seeded per plate	No. of plates	Average No. of plaques	Cells releasing virus
MP 4	125	5	101	1 in ~ 1
MP 10	400	2	98	1 in 4
	1,200	3	2	
MP 11	6,000	8	8	1 in 600–
	30,000	3	28	1 in 1,000

tumours tested, from fifteen mice, virus was obtained from every tumour. This is not the case in tumours induced by polyoma in other species.

The sarcomas induced in hamsters, and the fibromas (that eventually regress) induced in rabbits, are hard tissues, and good cell suspensions have so far not been obtained from them. Fourteen tumours from ten hamsters (7 subcutaneous sarcomas, 3 kidney sarcomas, and 4 heart sarcomas) have been tested for release of virus by placing minced pieces of tissue in Petri dishes either by themselves or on normal mouse embryo monolayers. No hæmagglutination titres higher than the control value for normal tissue culture fluid were found in the fluid of any of these cultures after 13 days, and second-passage cultures of six of these tumours have also given no hæmagglutination titres after 14 days. The fluids from eight tumours cultured for 13 days on mouse embryo monolayers were tested by the plaque assay, and of these seven gave no plaques, and one, a subcutaneous sarcoma, gave 180 plaque-forming units per ml. during a release period of six days. In the fourteen hamster tumours tested there was thus no, or almost no, release of virus from the tumour cells. The release of infectious virus is thus not necessary for the maintenance of malignancy.

Two rabbit fibromas from two rabbits were studied by placing minced pieces of tumour on mouse embryo monolayers. One tumour gave no hæmagglutination titre even after a second and a third passage of 14 days each. The other tumour gave no hæmagglutination titre in the first passage of 14 days, and a titre of 64 after 11 days in the second passage. The virus isolated from this rabbit has produced tumours in hamsters.

In rats, polyoma induces a high frequency of kidney sarcomas, especially after intracerebral inoculation[4], and these tumours give good cell suspensions by trypsinization. As with mice, 5×10^6 tumour cells were seeded with 4 ml. of medium, and the results for release of virus from four rat kidney sarcomas from four rats, cultured for periods ranging from 9 to 21 days, are shown in Table 2. No significant hæmagglutination titres were obtained in any of these cultures, and a plaque assay of the culture fluids has shown that at various times there was either no, or very low, release of virus. In contrast to a titre of $2{\cdot}8 \times 10^6$ plaque-forming units per ml. during a release period of 2 days obtained from mouse kidney sarcoma cells, the highest titre obtained from the rat kidney sarcoma cells was

Table 2. Release of Virus from Rat Kidney Sarcoma Cells in Culture

Tumour	Total plaque-forming units per ml.	Release period (days)
RK 1	20	9
RK 2	160	13
RK 3	90	17
RK 4	340	21

6×10^2 plaque-forming units per ml. during a release period of 3 days. Tests for inhibitors and antibodies in the rat culture fluids (and in the fluids from hamsters and rabbits) all gave negative results. Virus isolated from the rat sarcomas has produced tumours in hamsters.

Rat sarcoma *RK* 2 was further tested by determining the numbers of plaques produced by tumour cells placed on mouse embryo monolayers (4 plates per cell dilution). No plaques were obtained even with an inoculum of 10,000 cells. Thus a very small proportion of cells in the rat tumours (less than 1 in 10,000 in *RK* 2) release virus. It should be mentioned that *in vitro* infection of normal monolayers has shown that in contrast to the high rate of virus multiplication in mouse tissues, there appears to be no, or very little, multiplication of the same virus stock in monolayers of hamster, rat and rabbit cells.

The above experiments show that a genetically homogeneous line of polyoma virus can induce the formation of various tumours with different rates of release of virus, and in some tumours no release at all. It is significant that both for the Rous sarcoma[7] and for the Shope papilloma virus[8] it has also been shown that it is possible to induce either tumours from which virus can be readily recovered or tumours from which little or no virus can be extracted. The possibility of virus induction from a pro-virus state, the existence of immunity to super-infection, and the behaviour of single-cell clones of tumour cells releasing virus and not releasing virus is being studied. For such investigations the polyoma virus provides an unusually favourable system, since a plaque assay for the virus has been developed (the existence of non-infectious incomplete particles can be determined by hæmagglutination), and tumour cells with widely different cell–virus relationships, can be readily and regularly established and studied in culture.

We are indebted to the Winfield Baird Foundation for a grant in support of this work.

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EXPERIMENTAL DEMONSTRATION OF AN INTERNAL RHYTHM OF REPRODUCTION IN A TRANS-EQUATORIAL MIGRANT (THE SHORT-TAILED SHEARWATER *PUFFINUS TENUIROSTRIS*)

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AND

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THE trans-equatorial migration of the short-tailed shearwater or Australian 'mutton-bird' has been described and mapped by Serventy[1] (Fig. 1). Marshall and Serventy[2] further showed that the migratory flocks regularly leave their southern Australian breeding islands in April (autumn) at a time when their principal food—euphausian plankton—remains abundant, and when sea temperatures are still higher than at the November period of ovulation. It was concluded that the post-nuptial northern journey via Japan to the Aleutian and South Arctic contra-nuptial ('wintering') areas is probably in obedience to an inherent internal rhythm.

When the millions of birds first make their landfall at the breeding islands in the last week in September (spring), gametogenesis is already well advanced. The testes contain bunched spermatozoa. There is no doubt that both the spring sexual cycle and the southern journey are initiated under conditions of decreasing day-length while the birds are still north of the equator. However, the astonishing regularity of their Australian landfall and subsequent egg-laying (November 19–21 and the following 12 days) seemed to suggest that the nuptial journey must be 'timed' by some astronomical constant, possibly decreasing day-lengths. No obvious periodic changes that might operate as precise 'timing' factors were observed in the breeding area.

P. tenuirostris, in common with certain other long-distance migratory shearwaters, moults on the head and body shortly after egg-laying, but delays the replacement of its wing and tail quills until the completion of the exacting journey across the equator[3]. Like most other migrants the species lays down massive deposits of subcutaneous and peritoneal fat before migration.

The primary purpose of the present investigations was to try to determine whether decreasing day-length, or internal rhythm, controls the sexual cycle (and possibly, therefore, the migration). Secondly, it was desired to discover whether the peculiar moult rhythm would persist under varying stimuli. Thirdly, it was considered valuable to record the degree of fat deposition under such varying conditions in captivity.

A group of breeding adults was caught in the Furneaux group of islands in north-east Tasmania (lat. 40° 12′ S.) just before the beginning of the northward migration of 1957. They were sent by air to the Commonwealth Scientific and Industrial Research Organization Regional Laboratory at Perth, Western Australia (lat. 32° S.), and held in captivity for several months during the period they would normally spend in the North Pacific (see Fig. 1). It was found that after some initial losses those essentially plankton feeders could be maintained on a

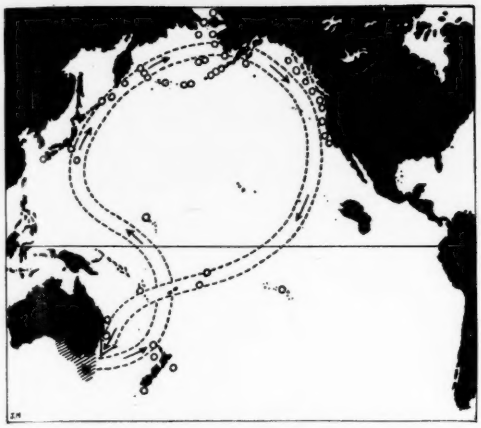

Fig. 1. Generalized migration route of *Puffinus tenuirostris*. The rings indicate localities of collected specimens. Arrows indicate the direction of migration. The breeding area is shown by means of parallel lines

diet of sliced fish. Experiments were conducted during two successive years.

Observations in 1957

The birds were divided into two groups of 15 individuals each. The first lot was placed under a changing light regime approximating the day-lengths to which travelling wild migrants should be exposed, except that the total period was telescoped by half. Thus, whereas the migrating flocks leave the breeding islands about mid-April and arrive back late in September, the artificial photo-periods in this experiment (successively equalling those of the equator, Japan, the Aleutians, the equator and finally the breeding localities) were so adjusted that the normal day-length of September 27 was reached on June 27.

The second group of birds was held under natural Perth daylight (which is longer at the June solstice by about an hour than at the breeding islands used by the species but almost 10 hr. shorter than at the Aleutians). Some of the birds from both cages were killed on June 27. The photostimulation experiment was then terminated and the survivors of both groups held in company, under ordinary Perth daylight, until September 29.

(*a*) *Gonad condition on June* 27. Despite the striking differences in the light regimes of the two groups of birds, there was no detectable difference in their gonads. Males of *both* groups showed some very slight advancement from the April condition and those that died during the first fortnight of the experiment. Thus, the seminiferous tubules of some birds contained up to three rows of spermatogonia. The diameter of ovarian oocytes in the single female killed was slightly greater than the April condition.

(*b*) *Gonad condition on September* 29. Four birds survived to this date. Of three males, one (originally from the photo-stimulated group) contained spermatozoa in some seminiferous tubules. Of the other two males (originally from the natural day-length cage) one was even more advanced, having bunched spermatozoa. The third male contained numerous primary spermatocytes in mitosis and a few secondary spermatocytes. The overall size of gonad was slightly, but definitely, smaller than normal wild birds at the corresponding time. A surviving female (originally from the photo-stimulated group) had the diameter of the largest oocyte measuring 3·1 mm.; that is, within the range of normal wild birds at the time of land-fall.

It was clear, then, that alteration of photo-periodicity, in the manner conducted in the experiment, had no great effect on the captive shearwaters. The birds—both experimental and control—that were allowed to survive came rhythmically into a sexual condition only a little less advanced than that of the wild population by the date that the latter completed their trans-equatorial migration around the Pacific.

Observations in 1958

The experiment was repeated with newly caught adults from the same breeding stations. Again they were divided into two groups. Half as before were kept under conditions of natural Perth daylight. The other half were retained under a constant 12 hr. illumination. This is the day-length at the breeding islands about the time of the exodus migration. This year birds were held until the period of normal landfall, when the survivors were killed and dissected on September 29. Three males and three females survived in the artificially lighted cage and seven males and two females in the natural light enclosure.

Again there was no appreciable difference in the sexual condition between the birds in the two cages. As before, all birds showed some gonad modification, and in some individuals testis development had almost, but not quite, reached the stage attained by normal wild birds at the corresponding time. Four out of the ten males had produced spermatozoa, and the remainder contained primary and sometimes secondary spermatocytes. The range of overall size reached that of the wild population, but the mean size was significantly below it. Ovarian development was also slightly advanced and the largest diameters of oocytes (mean, 2·1 mm.) were slightly inferior to them in normal birds at the corresponding stage.

Wing and tail moult began in late June and July, but the moult was protracted and its completion retarded by comparison with that of wild birds. Both groups of captives put on appreciable depot fat (corresponding to 'pre-migratory' fat) from mid-August onwards, reaching a maximum in early September. The weight trends were identical in the two groups.

In summary, these investigations show that in this migratory shearwater the breeding rhythm is persistent at least for one year after the animal is taken out of its normal environment and exposed to external influences (photo-period, temperature, food and habitat) exceedingly different from normal. Although gametogenesis, wing moult, and pre-migratory fat deposition took place under these conditions at approximately the normal periods, there was nevertheless in some individuals a considerable asymmetry of the seminiferous tubules and a pronounced retardation of spermatogenesis that is never encountered in the wild population. This suggests that internal events were tending to drift out of phase with the external environment and that essential (as yet undetermined) external influences, possibly including behaviour, were lacking.

The experiments are continuing.

[1] Serventy, D. L., Proc. Seventh Pacific Sci. Cong., **4**, 394 (1956).
[2] Marshall, A. J., and Serventy, D. L., *Proc. Zool. Soc. Lond.*, **127**, 489 (1957).
[3] Marshall, A. J., and Serventy, D. L., *Nature*, **177**, 943 (1957).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 14

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Why Hi-Fi?", opened by Mr. P. P. Eckersley.

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. P. B. Cornwell: "Insect Control by Gamma Irradiation".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Baron G. de Gerlache de Gomery: "The Belgian Antarctic Expedition".

Tuesday, December 15

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. K. Maddocks: "Some Aspects of Marine Reactor Security".

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. K. G. Freeman: "Tubes for Colour Television".

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Data Handling Problems in Atomic Installations", opened by Dr. D. Taylor.

Wednesday, December 16

POWDER METALLURGY JOINT GROUP OF THE IRON AND STEEL INSTITUTE AND THE INSTITUTE OF METALS (in the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m. —Symposium on "The Powder Metallurgy of Magnetic Materials".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. P. B. Ackermann and Prof. F. Walker: "Vitrification of Arkose by Dolerite near Heilbron, Orange Free State".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. H. Wexler: "Seasonal and other Temperature Changes in the Antarctic Atmosphere"; Mr. W. Hitschfeld: "Patterns of Hailstorms in Alberta".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Sir Hugh Beaver, K.B.E.: "Statistics as a Tool of Management" (Presidential Address).

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. C. Hartley: "Pipelines".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Dr. S. G. Hooker: "The Future of Air Breathing Engines in Aviation" (James Clayton Lecture).

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Discussion of Research Papers recently published by Members of the Group.

SOCIETY FOR ANALYTICAL CHEMISTRY, MICROCHEMISTRY GROUP (at "The Feathers", Tudor Street, London, E.C.5), at 6.30 p.m.—Discussion on "Applications of the Flask Combustion" opened by Miss M. Corner and Mr. C. B. Deanis.

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. F. Coales: "Random Inputs and Load Variations".

Thursday, December 17

BRITISH COMPUTER SOCIETY, LONDON BRANCH (at the Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 2.30 p.m.—Discussion on "Conversion between Analogue and Digital Representation".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m. and 5.30 p.m.—Scientific Papers.

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. J. Robinson: "Relationship Between Particle Size and Collector Concentration": Mr. D. Borchers: "Deepening of No. 1 Vertical Shaft at Premier (Transvaal) Diamond Mining Co., Ltd.".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. E. V. Schenkman: "Supersolvable Groups".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Presentation of the History and Philosophy of Science", opened by Prof. H. Dingle.

Thursday, December 17—Friday, December 18

PHYSICAL SOCIETY (in the Townsend Lecture Theatre, The Clarendon Laboratory, Oxford), at 10 a.m. daily—Conference on "Electron–Phonon Interaction in Solids".

Friday, December 18

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. F. E. Camps: "Some Aspects of Forensic Science".

Friday, December 18—Saturday, December 19

BRITISH PSYCHOLOGICAL SOCIETY (at University College, Gower Street, London, W.C.1)—Conference.

Saturday, December 19

BIOCHEMICAL SOCIETY (in the Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, S.E.5), at 10.30 a.m.—389th Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

KNOOP RESEARCH FELLOW (graduate in medicine or science), to work on cerebral enzymatic aspects and on the presence of chemical abnormalities in hereditary degenerative cerebral conditions—The Secretary, Institute of Neurology, The National Hospital, Queen Square, London, W.C.1 (December 18).

LECTURER or ASSISTANT LECTURER IN GEOGRAPHY at Makerere College (University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 30).

ASSISTANT LECTURER or LECTURER IN PURE MATHEMATICS, and an ASSISTANT LECTURER or LECTURER IN MATHEMATICAL STATISTICS —The Secretary, The University, Exeter (December 31).

LECTURER (preferably with an interest in the theory of machines, mechanical servomechanisms or the strength of materials) IN MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast (January 1).

PROFESSOR OF APPLIED MATHEMATICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (January 1).

LECTURER or ASSISTANT LECTURER IN CHEMISTRY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

ASSISTANT LECTURER IN STATISTICS, to teach theoretical statistics and some mathematics—The Secretary, The London School of Economics and Political Science, Houghton Street, Aldwych, London, W.C.2 (January 22).

ASSISTANT (preferably with some knowledge of horticulture and experience in proof correcting and indexing) TO THE EDITOR—The Secretary, The Royal Horticultural Society, Vincent Square, London, S.W.1, marking envelope "Confidential".

CHEMIST or AGRICULTURAL CHEMIST (with an honours degree in science or equivalent qualification, and preferably postgraduate research experience to Ph.D. level and a knowledge of agriculture), to undertake research on the quality of protein in animal feeding stuffs for non-ruminants—The Secretary, The Rowett Research Institute, Bucksburn, Aberdeen.

CHEMIST or BIOCHEMIST, for research in the steroid field—The Director, Group for Research on the Chemical Pathology of Steroids, Jessop Hospital for Women, Sheffield 3.

LECTURER (honours graduate in mathematics or mathematical physics) IN THE DEPARTMENT OF MATHEMATICS (including Industrial Statistics and Computing)—The Registrar, College of Technology and Commerce, Leicester.

PHYSICIST (with research experience to Ph.D. standard in infra-red spectroscopy), to undertake a programme of investigations in atmospheric radiation—Imperial College, Department of Meteorology, London, S.W.7.

RESEARCH ASSISTANT (with a good mathematical background, including statistical analysis, and preferably some knowledge of the field of metal fatigue) IN THE DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS, to undertake work on the theory of cumulative damage in fatigue—The Secretary and Registrar, The University, Southampton.

SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF GEOLOGY—The Bursar, The University, Sheffield 10.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Other Countries

Pacific Insects, Vol. 1, No. 1 (July 15, 1959). Pp. 172. (Organ of the Program "Zoogeography and Evolution of Pacific Insects.") Subscription rates, per volume (4 issues totalling at least 400 pages): Japan ¥ 1,700; N. America 5 Dollars; 35*s.* 6*d.* Single issues: ¥ 600; 2 dollars; 13*s.* (Honolulu, Hawaii: Entomology Department, Bernice P. Bishop Museum, 1959.) [2210

Inter-American Tropical Tuna Commission. Annual Report for the year 1958. Pp. 121. (La Jolla, Calif.: Inter-American Tropical Tuna Commission, 1959.) [2210

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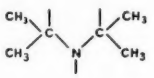
LETTERS TO THE EDITORS

BIOCHEMISTRY

Congeners of Pempidine with High Ganglion-blocking Activity

RESEARCH in these Laboratories on new hypotensive drugs has in recent years centred on the development of compounds exhibiting both a high level of ganglion-blocking activity and good absorption after oral administration. The first phase of this work culminated in the discovery of 1:2:2:6:6:-pentamethylpiperidine, pempidine[1]. In clinical use the good absorption and conveniently rapid excretion characteristics of this drug combine to make it a useful advance in the management of essential hypertension[2].

During the past two years we have examined some 150 congeners of pempidine, the great majority of which have contained the structural component :



Many of these compounds have exhibited a level of ganglion-blocking activity similar to that of pempidine in the nictitating membrane test in the anæsthetised cat and also a ratio of oral to intravenous toxicity in mice which indicated good absorption on oral administration. The results on these active compounds conformed to an interesting pattern (Table 1) which has enabled us to reach a number of tentative conclusions as to the structural requirements for activity in this series and it is our object here to discuss these conclusions.

In the first place we found, not unexpectedly, that when in congeners of pempidine, the nitrogen atom was substituted by groups which greatly reduced the basic strength, activity was largely eliminated, as in for example compounds (II, R = —NO) and (XII, R = —OH).

Secondly, it was soon apparent from the results on compounds (XII, R=H) and (XVI) that activity was not confined to derivatives of piperidine. Evidently, the over-riding factor controlling activity was not ring size but rather the shielding of the basic nitrogen atom provided by the alkyl groups substituted on the two carbon atoms adjacent to it. In the piperidine series (cf. particularly the results on XI, X and II, R=H) at least three methyl substituents in the 2- and 6-positions are required for activity. Among the pyrrolidines on the other hand four methyl substituents in the 2- and 5-positions are necessary for a useful level of activity (cf. XV and XII, R=H); examination of molecular models led us to conclude that the fourth of these methyl groups is required because the smaller bond angles in the pyrrolidine ring cause a reduction in shielding due to displacement of the methyl groups in the 2- and 5-positions away from the nitrogen atom, as compared with the spatial orientation of the corresponding methyl groups in pempidine. Corroboration that such an effect can influence activity is provided by the reduced (but still potentially useful) level of activity of (XIV) as compared with (XII, R = —C_2H_5); the double bond in (XIV) will evidently cause a further displacement of the methyl groups in the 2:2:5:5-positions away from the nitrogen atom. Moreover, we find that di-*tert*-butylamine (XVII), in which the ring is absent and α-C-methyl substitution is a maximum, has an activity similar to that of pempidine.

The introduction of various groups into the 1-position of 2:2:6:6-tetramethylpiperidine, giving compounds of structure (II), has further demonstrated the importance of the size and shape of the substituents surrounding the nitrogen atom. In its influence on activity, the chemical nature of the 1-substituent seems to be relatively unimportant so long as a sufficiently strong basic centre remains. The results do indicate, however, that there is a maximum size for the 1-substituent beyond which activity falls off rapidly. Molecular models of compounds of structure (II) all have roughly a hemispherical shape with the endocyclic nitrogen atom withdrawn a short distance from the centre of the planar face. So long as the 1-substituent attached to this nitrogen atom can be contained within this planar face the compounds are highly active. In (II, R = —$N(CH_3)_2$), one of the N-methyl groups must inevitably project somewhat from the planar face, and one can speculate that the low activity of this compound is related to the fact that the projecting methyl group prevents the molecule sitting squarely on a receptor site.

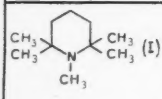
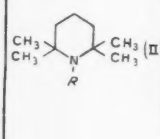
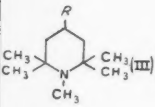
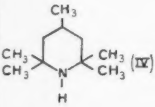
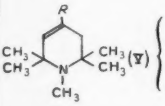
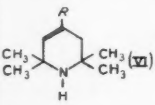
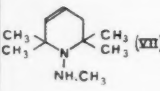
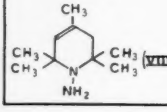
The observed activity of (II, R = —C_2H_5, R = —$NH.CH_3$ and R = —$N{=}CH.CH_3$) at first sight clashes with this speculation because in the probable favoured configuration of these three molecules an alkyl group projects from the planar face of the outline of molecular models. However, in contrast to (II, R = —$N(CH_3)_2$) these three molecules are capable of assuming configurations in which the alkyl groups in question fall *within* the planar face and one can suppose that this, in fact, happens on approach to the receptor site.

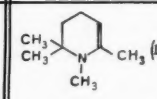
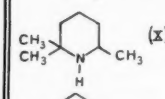
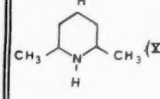
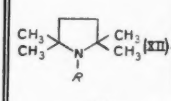
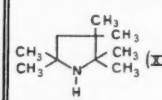
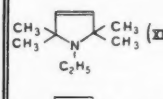
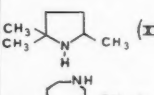
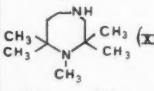
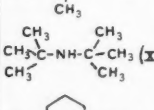
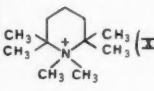
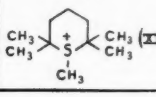
In general, we find that the introduction of a 3:4 or a 2:3 double bond into pempidine and its active congeners does not materially affect their activity; it may be relevant that this modification does not alter the general hemispherical shape of the molecular models. When, however, this shape is profoundly altered by the introduction of substituents into the 4-position, it has proved difficult to relate structure to activity, but as can be seen from Table 1, widely varied groups can be introduced into this position without adverse effect on activity (cf. compounds III and V).

Conversion of pempidine to its quaternary salt (XVIII) increased its ganglion-blocking activity but greatly diminished its duration of action in the nictitating membrane test; moreover, as expected, the compound had a high oral to intravenous toxicity ratio in mice. As has been frequently observed in the case of other quaternary ganglion-blocking agents, the sulphonium analogue (XIX) of (XVIII) also had an appreciable level of activity.

It cannot be inferred that there will be a close quantitative correlation between ganglion-blocking activities determined in the present work by a specific test in a single species, and hypotensive

Table 1. Activity on the Pre-Ganglionically Stimulated Nictitating Membrane of the Cat and Toxicity in the Mouse of 34 Congeners of Pempidine

Structure of compound	Activity potency[a]	Activity duration[b]	LD50 (mgm/kgm) i.v.	LD50 (mgm/kgm) oral
 (I) Pempidine	1·35[c]	long	39·5	275
 (II) R = H	2·0[c]	long	32·5	220
R = $-C_2H_5$	2·0[c]	long	24·4	148
R = $-NO$	0·04	long	25·0	300
R = $-NH_2$	1·4	short	44·3	261
R = $-NHCH_3$	1·2	long	25·0	235
R = $-N(CH_3)_2$	0·03	long		
R = $-N{=}CH_2$	1·75	long	57·0	396
R = $-N{=}CH.CH_3$	1·0	long		
 (III) R = Cl	1·0	long		
R = Br	1·0	short	51·0	172
 (IV)	1·0	long	17·7	127
 (V) R = H	2·3	long	79·0	460
R = $-C_2H_5$	1·0	long	13·2	82
R = $-(CH_2)_3N(CH_3)_2$	1·0	medium	41·0	460
R = $-C{\equiv}CH$	1·0	medium	30·0	330
 (VI) R = H	1·0	long	34·0	230
R = $-CH_3$	1·0	short	58·0	165
 (VII)	1·0	medium		
 (VIII)	1·0	short		

Structure of compound	Activity potency[a]	Activity duration[b]	LD50 (mgm/kgm) i.v.	LD50 (mgm/kgm) oral
 (IX)	1·4	short	40·0	310
 (X)	1·4[d]	short	53·8	210
 (XI)				
 (XII) R = H	0·5	long	63·0	450
R = $-CH_3$	0·5	long	72·0	316
R = $-C_2H_5$	2·0	long	51·0	360
R = $-C_3H_7$	0·5	long	30·4	200
R = $-OH$	0·025	medium		
 (XIII)	1·0	long		
 (XIV)	0·75	medium		
 (XV)	0·05	very short		
 (XVI)	0·4	short	99·0	1970
 (XVII)	1·4	long		
 (XVIII)	3·0	very short	11·0	186
 (XIX)[f]	1·0	very short	6·55	140

a, Hexamethonium = 1·0.
b, Short = similar to hexamethonium ; long = similar to pempidine.
c, Spinks *et al.* (1958)[1] report activities of 2·5, 2·0 and 3·0 times mecamylamine for (I), (II, R = H) and (II, R = C_3H_4) respectively.
d, Spinks *et al.* (1958)[1] report an activity of 0·4 times mecamylamine.
e, Spinks *et al.* (1958)[1] report this compound to be devoid of activity : Gyermek and Nádor (1957)[3] report an activity of 2·0 times tetraethylammonium bromide for the *N*-methyl derivative.
f. Prepared by the literature method[4].

activity in man. Many other factors including the incidence of side effects will determine the therapeutic value of these compounds. However, exploratory clinical study of selected members of this series has produced some interesting results.

We thank Dr. H. J. Barber and Dr. R. Wien for the constructive interest they have taken in this work.

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Localization of β-Glucuronidase in Normal and Cancer Cells

It has been shown by Walker[1] that in a mouse-liver homogenate in isotonic sucrose solution by far the greater part of the β-glucuronidase activity sediments in the cytoplasmic granules (mitochondria and microsomes). It was concluded that little, if any, of the enzyme is present in the nuclei or free in the cytoplasm. This was also true of rapidly growing or regenerating liver. Within the granules the enzyme displayed only fractional activity due to its inaccessibility to substrate. In these respects β-glucuronidase, in rat liver at least, resembles a number of hydrolytic enzymes, which, it has been suggested, are enclosed together within a special type of subcellular particle, the lysosome[2].

Levvy[3] has shown that β-glucuronidase is powerfully inhibited by saccharo-1:4-lactone which causes marked and specific inhibition at concentrations of the order of micromoles. This inhibitor has been employed by oral administration to arrest liberation in the bladder of carcinogens from their glucuronides by urinary β-glucuronidase[4]. Because of its location within the granules in liver cells it has been suggested[2,4] that β-glucuronidase is virtually inactive in healthy tissues and only displays its action when free in body fluids like urine.

It was considered that other tissues might display a pattern of intracellular distribution different from that in liver. Results for mouse kidney and *S*37 subcutaneous tumours compared with those for liver are shown in Table 1. In the scheme of fractionation adopted, in the case of liver[1], fraction I comprises nuclei and unbroken cells, whereas fractions II and III contain the cytoplasmic granules, and fraction IV unsedimentable enzyme in the cytoplasm. From the work of Straus[5] it would appear that fractions I and II in the case of kidney may also contain 'droplets' that possess β-glucuronidase activity.

Table 1. Intracellular Distribution of β-Glucuronidase in Various Mouse Tissues

Tissues were homogenized in 0·25 *M* sucrose in a glass homogenizer (1,500 rev./min.). Fractionation was carried out as described by Hogeboom (ref. 9). Results are expressed as a percentage of the total activity of the homogenate, all assays being done in presence of 0·1 per cent 'Triton X-100' (ref. 1). In columns *A*, the tissue was homogenized for 1 min. before fractionation. In columns *B*, fraction I was separated at intervals and rehomogenized, the supernatant fluids being combined for further fractionation.

	Liver	Kidney		*S*37 Tumour	
	(*A*)	(*A*)	(*B*)	(*A*)	(*B*)
Fraction I	6	32	17	23	11
Fraction II	26	22	71	15	22
Fraction III	55	21		13	
Fraction IV	12	21	16	41	57

Under similar conditions of homogenization, a larger proportion of the enzyme is found free in the cytoplasm of kidney and tumour than in that of liver. Moreover, both kidney and tumour differ from liver in their rate of breakdown in 0·25 *M* sucrose, as can be seen from the much larger proportion of the enzyme remaining in fraction I after homogenizing for 1 min. Prolonged homogenization of both tissues reduces the percentage activity in fraction I to values approaching those for liver, but fractions II and III also fall in activity, indicating breakdown of the particles. If fraction I is repeatedly removed and rehomogenized separately for short periods, there is a marked difference in behaviour between the kidney and tumour preparations. In the case of kidney, the bulk of the enzyme then passes into fractions II and III, and it is evident that fraction IV of the original homogenate is almost entirely derived from the particles. The tumour, in contrast to the normal tissues, shows equally rapid liberation of enzyme into both the cytoplasmic particles and the cytoplasm, and it is concluded that about half the enzyme in the tumour cells is initially free in the cytoplasm. In the tumour the total β-glucuronidase activity is high, being comparable with that of liver and kidney[6].

It has been reported that much of the β-glucuronidase activity in rat spleen is free in the cytoplasm[7], and that feeding azo dyes to rats causes the release of a very small proportion of the enzyme from the cytoplasmic granules in rat liver[8].

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Enzymic Reduction of α-Aminoadipic Acid by Yeast Enzyme

Recent work in this laboratory demonstrated that, with the whole cells of *Torulopsis utilis* and baker's yeast, α-aminoadipic acid is converted to lysine to an appreciable extent[1] and that it has a primary role for lysine biosynthesis in this organism, as inferred earlier by Strassman and Weinhouse[2]. Enzymic conversion of α-aminoadipic acid to lysine has so far been supposed to occur via α-aminoadipic acid-δ-semialdehyde, which actually has been shown to be an oxidation product of hexahomoserine catalysed by mould enzyme[3]; but no evidence for the enzymic reduction of α-aminoadipic acid to its semialdehyde has hitherto been presented. The present communication reports the enzymic reduction of α-aminoadipic acid to an intermediary substance which is easily converted to dehydropiperidine-2-carboxylic acid, a cyclized form of α-aminoadipic acid-δ-semialdehyde.

The enzyme solution was prepared by grinding a frozen cell paste of baker's yeast (or *T. utilis*) with glass wool powder and extracting with 0·05*M* potassium

Table 1. Requirements for the Enzymic Reduction of α-Aminoadipic Acid

	Complete	Complete minus : α-AA	TPN	ATP	GSH	Mg^{++} Mn^{++}	*Iso*-citrate	Enzyme fraction I	II	I & II
Optical density at 480 mμ	0·295	0·017	0·035	0·007	0·120	0·148	0·250	0·016	0·080	0·017

The complete system consisted of 0·51 mgm. of enzyme fraction I, 0·22 mgm. of enzyme fraction II, 6 μmoles of DL-α-aminoadipic acid (AA), 2 μmoles of adenosine triphosphate (ATP), 2 μmoles of *iso*citrate (DL+allo), 2 μmoles of $MgCl_2$, 0·09 μmoles of $MnCl_2$, 0·02 mgm. of triphosphopyridine nucleotide (TPN), 80γ of glutathione (GSH) and 0·24 ml. of 0·05*M* barbital buffer (*p*H 8·0) to a total volume 0·4 ml. The mixtures were incubated for 2·5 hr. at 30°

phosphate buffer (*p*H 7·2), occasionally adding dilute ammonia. After removal of insoluble protein of the extract at *p*H 5, the crude enzyme solution was separated into two fractions, enzyme fraction I and II. Enzyme fraction I is obtained between 33 to 50 per cent saturation with ammonium sulphate and the fraction II between 50 and 100 per cent saturation. Furthermore, enzyme fraction I was purified tenfold by subfractionation with ammonium sulphate. It has been proved that enzyme fraction II can be replaced by reduced triphosphopyridine nucleotide in the reaction.

For the determination of the reduction product of α-aminoadipic acid a simple method was devised ; to the reaction mixtures, after adjusting to *p*H 6·5–7·0 with 0·5*M* phosphate buffer, was added 0·4 ml. of 2 per cent *p*-dimethylaminobenzaldehyde in 'Methylcellosolve'. This was followed by heating for 20 min. in a boiling water-bath. After an orange-yellow colour had developed, the solutions were diluted to 3 ml. with 60 per cent ethanol and the precipitate was centrifuged down. The colour intensity of the supernatant was read on a Beckman *DU* spectrophotometer at 480 mμ. Typical results are presented in Table 1. For the reduction of α-aminoadipic acid in this system, the following cofactors were required ; adenosine triphosphate, triphosphopyridine nucleotide, *iso*citric acid, magnesium and manganese ions and a sulphydryl compound such as glutathione or cysteine. The reaction was optimal at *p*H 7·6–8·0. Enzyme fraction I was completely inhibited by monoiodoacetamide or *p*-chloromercuribenzoate at a concentration of $2{\cdot}5 \times 10^{-3}M$ and quite specific in its requirement for reduced triphosphopyridine nucleotide. Of eleven amino-acids tested including homoglutamine, α-aminoadipic acid-δ-hydrazide, acetyl-α-aminoapidic acid, piperidone carboxylic acid, piperidine-2-carboxylic acid and lysine, α-aminoadipic acid was the only active substrate in this system.

The reduction products of α-aminoadipic acid were isolated from the acid by means of 'Dowex-50' (0·9 cm. × 16 cm., RH type) or 'Amberlite IR-112' chromatography. The substances were detected by the orange-yellow colour formed with *p*-dimethylaminobenzaldehyde on paper or in solution. After incubation the reaction mixture was brought to *p*H 1 with hydrochloric acid, and deproteinized with ethanol (final concentration 50 per cent). The supernatant was mounted directly on a column of 'Dowex-50' and eluted successively with 18 ml. (3 portions of 6 ml. each) of 1·5*N*, 2*N*, 3*N*, 4*N*, and 6*N* hydrochloric acid. Aliquots of each eluate were determined with *p*-dimethylaminobenzaldehyde after removal of hydrochloric acid *in vacuo*.

On prolonged incubation (about 20 hr.), the substance giving a positive reaction with *p*-dimethylaminobenzaldehyde appeared in both the 3*N* and 6*N* hydrochloric acid eluates, while with a short-period incubation (about 3 hr.) a large part of the substance was eluted with 6*N* hydrochloric acid. The substance in the 3*N* hydrochloric acid eluate giving a positive reaction was found to have similar properties to dehydropiperidine-2-carboxylic acid prepared by the method of Meister[4].

On the other hand, the substance in 6*N* hydrochloric acid eluate giving a positive reaction with *p*-dimethylaminobenzaldehyde had an R_F value of 0·08 on paper chromatogram (*n*-butanol : acetic acid : water/8 : 1 : 2 v/v) and gave no piperidine-2-carboxylic acid or hexahomoserine on reduction with platinum oxide or Raney nickel catalyst even at a pressure of 50 atmospheres of hydrogen. However, when its aqueous solution (*p*H 3–4) was heated in a boiling water-bath for 10 min. or stood at room temperature for a long time and rechromatographed on 'Dowex-50' as above, most of the substance giving a positive reaction with *p*-dimethylaminobenzaldehyde appeared in the 3*N* hydrochloric acid eluate, and it also had similar properties to dehydropiperidine-2-carboxylic acid.

Hence it is probable that the substance in the 3*N* hydrochloric acid eluate giving a positive reaction with *p*-dimethylaminobenzaldehyde is a cyclized structure of α-aminoadipic acid-δ-semialdehyde and that in 6*N* hydrochloric acid is a derivative of α-aminoadipic acid-δ-semialdehyde (non-cyclized form). Their precise structures are under investigation.

Full details of this work will be published elsewhere.

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Gas Chromatographic Separation of Long-chain Fatty Acid Methyl Esters on Polyvinyl Acetate

Polyester-type partitioning media for the separation of mixtures of the methyl esters of long-chain saturated and unsaturated fatty acids by gas chromatography have recently been reported[1,2,3]. Some of these materials tend to 'bleed' excessively at the temperatures required for the separation of the fatty acid esters. In addition, polyesters from the same polyglycols and dibasic acids, when prepared in different laboratories, may have dissimilar properties since the average molecular weight, as well as the distribution of molecular weight in the final polymer mixture, is not adequately controlled.

We wish to report on the use of commercially available polyvinyl acetates to separate completely the methyl esters of stearic, oleic, linoleic, and linolenic acids. These naturally occurring C_{18} acids are those least amenable to separation.

The thermal degradation of polyvinyl acetate has been studied[4]. The products of degradation are acetic acid and polyvinylacetylene. Acetic acid reportedly becomes detectable at 210°C. In preliminary stability studies we found that for a freshly prepared column at a helium gas flow of 100 ml./min. at 195°C. the amount of acetic acid formed was less than 0·1 mgm./hr. per gram of polyvinyl acetate. At 205°C. acetic acid was evolved at the rate of 0·5 mgm./hr. per gram of polyvinyl acetate ; at 230°C. this rate

was 1·5 mgm./hr. of acetic acid per gm. of polyvinyl acetate. This rate of acetic acid evolution decreased with time without impairing the efficiency of the column. In all studies the base line drift was less than ±·02 mV. The infra-red spectra of the products collected at 230°C. over a 12-hr. period indicated the presence of acetic acid only. Thus, if the fatty acid methyl esters separated by the use of a polyvinyl acetate column are collected for further study, any trace impurities from the column can be readily removed, or easily compensated for in spectrophotometric studies.

The results reported in Table 1 were obtained using a polyvinyl acetate polymer of a rather low degree of polymerization with a molecular weight of approximately 1500. Similar results were obtained with a polyvinyl acetate polymer of molecular weight of approximately 23,000.

TABLE 1

Compound	Retention vol. (ml.)*
Methyl palmitate	1080
Methyl stearate	1860
Methyl oleate	2100
Methyl linoleate	2350
Methyl linolenate	2750
Methyl arachidonate	5230

* Retention volumes calculated for a flow-rate of 83 ml./min. and measured from the time of emergence of the solvent phase.

An 8-ft. coiled copper column, ¼ in. outside diameter and wall thickness 0·03 in., was used. The partition medium consisted of 15 per cent polyvinyl acetate, designated as 'Vinylite *AYAC*'[5], of a molecular weight of approximately 1500 on 'Chromosorb', 30–60 mesh[6]. The column packing was prepared by slurrying the 'Chromosorb' with a 10 per cent solution of the polymer dissolved in acetone. The acetone was evaporated at room temperature and stray volatile materials removed by heating in a vacuum oven at 130°C. The gas chromatograph was a commercial model[7] provided with a 1-mV., 1-sec. full-scale strip-chart recorder[8]. The detector was a 4-filament thermal conductivity unit. The helium flow rate was 83 ml./min. measured at the column exit and at room temperature. The column and cell temperature was 205°C. The pressure drop across the column was 30 p.s.i.g. Two mgm. of each methyl ester were dissolved in petroleum ether (b.p. 37°) and a 5-μl. sample injected into the column. Values for methyl palmitate and methyl arachidonate are included in Table 1 for comparative purposes.

Quantitative estimation of the fatty acid esters within ±5 per cent could be made by measuring the peak areas and comparing these values with those obtained from known amounts of the pure methyl esters.

This communication is taken from a dissertation submitted by Irwin Hornstein to the Graduate School of Georgetown University in partial fulfilment of the requirements for the Ph.D. degree.

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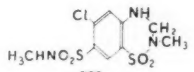
[7] A Beckman GC-2 gas chromatograph was used.
[8] The recorder was manufactured by Minneapolis-Honeywell, Brown Instrument Division, Philadelphia, Pennsylvania.

Sulphonamides with Diuretic Activity

RECENTLY we have reported that sulphonamides with a substituted amido-group that is, compounds deprived of any inhibitory action on carbonic anhydrase, may possess a high diuretic activity[1]. The most active compound of the series is 4-amino-6-chloro-benzene-1,3-disulphonmethylamide (I).

In the meantime cyclic compounds with a free sulphonamido-group, for example, 6-chloro-7-sulphamyl-3, 4-dihydro-1, 2, 4-benzothiadiazine-1, 1-dioxide (II), have received attention[2].

It seemed therefore interesting to us to bring about ring-closure with formaldehyde in compound (I), and 6-chloro-7-methylsulphonamide-3, 4-dihydro-2, N-methyl-1, 2, 4-benzothiadiazine-1, 1-dioxide (III) was obtained.



III

m.p. = 202–203°

(Anal. calc. for $C_9H_{12}ClN_3O_4S_3$: C, 33·28; H, 3·71; N, 12·81; S, 19·68; found: C, 33·20; H, 3·88; N, 12·62; S, 19·87)

The diuretic activity of compound (III) is of the same magnitude as that developed by (II) although it does not present an inhibitory action on carbonic anhydrase. Furthermore in low dosages it is possible to provoke diuretic activity much more pronounced than that observed with compound (I).

On the other hand between compounds (II) and (III) there exist considerable differences. While the diuresis promoted by (II) is a rapid phenomenon and the activity decreases after a short time, diuresis promoted by compound (III) is milder and more prolonged. Moreover (III) proves to possess a lower toxicity than (II): (DL_{50} intrap. in rats: (II) = 800 mgm./kgm.; (III) = 1,670 mgm./kgm.).

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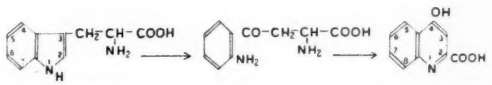
Specificity of the Tryptophan Peroxidase-Oxidase Enzyme System

THE conversion of tryptophan to kynurenic acid has been demonstrated in most mammals[1,2].



Tryptophan Kynurenine Kynurenic Adid

The formation of kynurenine (via formyl-kynurenine), is catalysed by a coupled peroxidase-oxidase system which is regarded by Hayaishi[3] and others, from *in vitro* experiments, as being highly specific for L-tryptophan.

The formation of kynurenic acid follows removal of the aliphatic alpha amino-group by a transaminase[4], which is, however, not so specific as the tryptophan peroxidase-oxidase, and will catalyse transamination of molecules substituted in the benzene nucleus[5].

The experiments to be described demonstrate the conversion of methoxytryptophans into kynurenic acid derivatives *in vivo*. 5-Methoxy and 7-methoxy tryptophan[5], were administered separately to a rabbit by subcutaneous injections. Chromatographic examination of the urine, following the injections, gave strong evidence for the formation of 6-methoxy and 8-methoxy kynurenic acids from these two substances, respectively. The chromatographic examination of the urine samples was carried out after treatment by the Capaldi procedure for precipitating kynurenic acid. Since no precipitate was obtained, the acidified concentrate was applied to one-dimensional chromatograms run by the ascending or descending techniques. The solvents used were *n*-butanol saturated with water; *n*-butanol, acetic acid, water (4:1:5); and methanol, *n*-butanol, benzene, water (2:1:1:1). A control spot consisting of the sample under investigation with added synthetic 6-methoxy and 8-methoxy kynurenic acids[7], was applied to each chromatogram. These two kynurenic acid derivatives could clearly be seen on the final chromatogram by examination under ultra-violet light, since they fluoresce very intensely with a purple and a blue colour respectively. Clear-cut results were obtained with all three solvent systems, confirming as conclusively as is possible by chromatographic means alone, the metabolic conversions already indicated.

The blue fluorescent spots from 45 butanol-water chromatograms of the urine concentrate following administration of 7-methoxytryptophan (0·6 gm./kgm.), were extracted and re-applied to a chromatogram run with methanol, *n*-butanol, benzene, water. A photograph of this chromatogram is shown in Fig. 1. The four spots cut out, which were derived from the major part of the above extract, were again extracted, in an attempt to plot the characteristic ultra-violet absorption spectrum for 8-methoxykynurenic acid. The curve so obtained proved impossible to identify, due to the presence of other substances. The spot still remaining on the chromatogram can clearly be seen alongside the control in Fig. 1, illustrating the identical R_F value of the unknown, with that of 8-methoxykynurenic acid.

An important implication of these results, is that the tryptophan peroxidase-oxidase system is not as specific, at any rate in the rabbit, as has hitherto been supposed[3,8,9]. Most of the available evidence for the specificity, has been obtained, however, with cell free preparations from bacteria[3,8]; the only published observation with mammalian enzymes involved 5-hydroxy and 7-hydroxy tryptophan, and appear to have been carried out by Mehler[3,10]. It may be, therefore, that the mammalian enzyme is less specific than the bacterial one or, alternatively, the whole animal may be capable of oxidizing substances not oxidized by the partially purified liver enzyme.

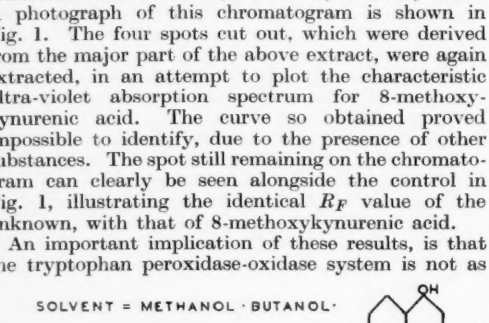


Fig. 1.

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Heparin and Cellular Calcium

It has been suggested, on the basis of indirect evidence, that heparin may extract calcium from cells[1].

We made a direct test of this hypothesis, using as our material Ehrlich ascites tumour cells of mice. Heparin is not usually necessary to prevent the ascitic fluid from clotting, but some investigators routinely add this or other anticoagulating agents to their preparations. Therefore, it is of interest to find if heparin affects the cells.

For these experiments we used cells labelled for 20 min. *in vivo* with the isotope calcium-45. We have reason to suppose that after this time-interval all, or almost all, the cell calcium is uniformly tagged[2]. Measurement of the radioactivity present in the cells is therefore a very sensitive indicator of the calcium content.

The tumours were grown for 9 days in male C3H mice. Calcium-45 of high specific activity was injected intraperitoneally, and after 20 min. the tumour was tapped off into polythene containers. The heparin (Evans, London; potency 106 units per mgm.) was dissolved in saline as a concentrated solution, and a very small volume of this was added to 1 ml. or 2 ml. of the tumour to give a final concentration of 0·2, 0·4 or 0·6 mgm. per ml. To the control samples the same volume of normal saline was added. The cells were then incubated at 37° for 20 min. The containers were rotated rapidly enough to keep the cells in suspension during incubation.

At the end of the incubation the cells were spun down at 3,500 r.p.m. for 5 min. Supernates and sediments were separately mineralized by a wet ashing procedure, and assayed for calcium-45 by a technique described elsewhere[2].

Table 1
Counts per minute of calcium-45 per gm. wet weight (Mean of two samples)

Concentration of heparin in mgm./ml.	Supernates		Sediments	
	Heparin treated	Control	Heparin treated	Control
0·2	302,500	302,250	112,300	110,600
0·4	244,500	252,100	84,300	85,150
0·6	287,000	290,500	94,300	102,800
	182,800	182,000	55,200	57,700
	225,500	221,000	70,600	69,400
	207,500	199,600	71,200	67,000

The results are shown in Table 1. If these results are worked out as the ratio calcium-45 of heparin-treated sediment/calcium-45 of control sediment then the mean ratio with standard error is $0{\cdot}99 \pm 0{\cdot}02$. The corresponding ratio for supernates is $1{\cdot}00 \pm 0{\cdot}01$.

We conclude from these results that at concentrations of up to 0·6 mgm. per ml., and under these conditions, heparin does not extract calcium from Ehrlich ascites tumour cells, or, in fact, from the mouse leucocytes which to a greater or lesser extent contaminate these tumours.

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Monosaccharide Transport in Rat Diaphragm Muscle

THE passage of glucose across the muscle cell membrane appears to be a major site for regulation of glucose uptake in this tissue. Accelerating effects on glucose uptake both of insulin and of factors such as anoxia which inhibit oxidative phosphorylation appear to result in skeletal and cardiac muscle from an enhanced rate of transfer of the sugar across the muscle cell membrane[1-4]. Recent studies[5] have emphasized that the movement of sugars across the cell membrane is predominantly a transport process, that is, it involves combination of the sugar with a particular constituent of the cell. In the present investigation we have sought to define the specificity of monosaccharide transport systems in diaphragm by determining which monosaccharides compete for transport and to obtain information about the nature of the system by studying the effect of certain enzyme inhibitors on transport of monosaccharides.

The rate of transport has been assessed by measuring the accumulation of non-utilizable sugars in diaphragm muscle after 30-min. of incubation (during which time accumulation is not complete). Except in experiments marked thus * (where only the sugar space was measured) the intracellular accumulation of sugar was calculated by subtracting the volume of extra-cellular fluid (measured as the sorbitol space with D—[$^{14}C_6$]-sorbitol) from the sugar space of the tissue. In studying competition the effect of other sugars on the accumulation of a particular monosaccharide was compared with that of a similar concentration of D—[^{12}C]-sorbitol (a compound with no influence on monosaccharide transport). Competition is deemed to occur if the intracellular accumulation of one sugar is reduced by the presence of another sugar. The procedure was as follows. Intact diaphragms[3] from male or female albino Wistar rats weighing 100–150 gm. were incubated at 37° for 30 min. in 5–15 ml. of a bicarbonate buffered salt solution[6] gassed with oxygen + carbon dioxide (95:5) containing D—[$^{14}C_6$]-sorbitol (0·12 μc./ml.); the sugar the transport of which was being studied (8·3 mM) and either D—[^{12}C]-sorbitol or the sugar the influence on transport of which was being investigated (26 mM). When insulin was present its concentration was 0·1 unit/ml. After incubation the muscle was extracted by boiling in 3 ml. of water for 10 min. The extract and samples of medium suitably diluted were then deproteinized with zinc hydroxide[7], and assayed for [^{14}C] and sugar (for methods, definition of concept of space and calculation of spaces see refs. 3 and 4).

The intracellular D-xylose space (in ml./100 gm. of wet muscle) was 7 in the absence and 24 in the presence of insulin. In the absence of insulin, xylose accumulation was diminished by D-glucose (mean ± *S.E.*-3 ± 1) and by D-3-*o*-methyl glucose ($-5 \pm 1{\cdot}4$*). In the presence of the hormone accumulation of xylose was diminished by D-glucose ($-6 \pm 1{\cdot}7$), D-mannose ($-7 \pm 1{\cdot}3$) and D-3-*o*-methyl glucose ($-3 \pm 1{\cdot}1$) but not by D-galactose ($1 \pm 1{\cdot}3$), D-fructose ($-3 \pm 2{\cdot}2$*) or α-methyl-D-glucoside ($0 \pm 1{\cdot}4$) or maltose ($1 \pm 1{\cdot}4$). The intracellular L-arabinose space in the presence of insulin was 21 ml./100 gm. and this was diminished by D-glucose (-12 ± 2), D-mannose ($-11 \pm 1{\cdot}8$), and D-3-*o*-methyl glucose ($-12 \pm 1{\cdot}7$*) but not by D-galactose, D-fructose or α-methyl-D-glucoside ($3 \pm 3{\cdot}4$, $-3 \pm 2{\cdot}2$ and $2 \pm 2{\cdot}3$ respectively). The intracellular D-arabinose space (10 ml./100 gm. in the presence of insulin) was diminished by D-glucose ($-3 \pm 1{\cdot}1$). The intracellular D-galactose space (30 ml./100 gm. in the presence of insulin) was not reduced by D-glucose ($8 \pm 5{\cdot}6$). In further experiments the effect of D-glucose on the accumulation of D-xylose and of D-3-*o*-methyl glucose on the accumulation of D-lyxose was measured at 10, 20, 30 and 40 min. of incubation. The accumulations of D-xylose were diminished by the presence of D-glucose (−3, 10, 12 and 17 ml./100 gm. respectively) and of D-lyxose by the presence of D-3-*o*-methyl glucose (−12, 9, 13 and 13 ml./100 gm. respectively).

The transport of D-mannose in diaphragm cannot be assessed by measuring intracellular accumulation of the sugar, because mannose is rapidly metabolized by diaphragm muscle. The rates of uptake and of incorporation of [^{14}C] from D—[1-^{14}C]-mannose into glycogen and maltose have therefore been used as parameters of the transport-rate for this sugar. In the presence of insulin the uptake of D—[^{14}C]-mannose (isolated from the medium for analysis by paper chromatography) was 767,000 counts/min./gm. of wet muscle/hr. of incubation in the absence of D—[^{12}C]-glucose but only 430,140 when glucose was present. In a further experiment the incorporations into glycogen and maltose were 486,714 and 1880 counts/min./gm./hr. respectively when glucose was omitted and 288,267 and 956 when glucose was present (concentrations: D—[^{12}C]-mannose, 0·3 mgm./ml.; D—[1-^{14}C]-mannose 1·3 μc./ml.; D—[^{13}C]-glucose 3 mgm./ml.).

The effects of phloridzin (3 mM), *p*-chloromercuribenzoate (10 μM) N-ethylmaleimide (mM) and diisopropylfluorophosphonate (20μM) on the transport of D-xylose in diaphragm was also investigated. The intracellular lyxose space was reduced by phloridzin ($-15 \pm 3{\cdot}8$), *p*-chloromercuribenzoate ($-4 \pm 1{\cdot}3$) and N-ethylmaleimide ($-20 \pm 1{\cdot}7$) but not by diisopropylfluorophosphonate ($-1 \pm 3{\cdot}1$).

We conclude from these observations that members of the following group of sugars compete for, and are therefore transported by, the same system: D-glucose, D-mannose, D-3-*o*-methyl glucose, D-xylose, D-lyxose, D-arabinose and L-arabinose. Since competition could not be demonstrated between members of this group and D-galactose and D-fructose (sugars which penetrate muscle cells of diaphragm when insulin is present) it is concluded that D-galactose and D-fructose must be transported by a different system or systems. Maltose and α-methyl-D-glucoside neither compete for transport nor do they penetrate the muscle cells of diaphragm, and it is concluded that

they cannot combine with constituents of the cell responsible for monosaccharide transport. The structural features of these sugars suggest that the following factors are of importance to the specificity of monosaccharide transport systems in diaphragm muscle: (1) ketose or aldose structure since the only ketose tested (fructose) is transported by a different system from that which transports most of the aldose sugars studied; (2) a methoxy group on C_5 of the pyranose ring is not necessary for transport because pentoses and hexoses are transported by the same system; (3) our observations with 3-methyl glucose and those of Kipnis and Cori[8] with 2-deoxyglucose show that a hydroxyl on C_2 or C_3 of the pyranose ring is not necessary; (4) since α-methyl-D-glucoside neither competes nor is transported a free hydroxyl on C_1 of aldose sugars may be essential; alternative possibilities are that a methyl group in this position interferes with transport or that a hydroxyl or methoxy group on C_1 of aldose sugars must be in the β-position; (5) the positions of hydroxyl groups on C_2, C_3, C_4 or C_6 in relation to the plane of the pyranose ring are not determinant except that, (6) the relative positions of hydroxyl groups on C_4 and C_6 may determine whether an aldose sugar is transported by one system or another because galactose which is unique amongst sugars tested in having hydroxyl groups on C_4 and C_6 on the same side of the plane of the pyranose ring did not compete for transport with the other aldose sugars tested.

Transport of D-xylose was not inhibited by diisopropylfluorophosphonate thus excluding participation of enzymes or amino-acid sequences reacting with this substance (for review see ref. 9). On the other hand, transport of xylose was inhibited by the —SH poisons *p*-chloromercuribenzoate and N-ethylmaleimide. This suggests either that transport involves combination of sugar with an —SH compound and perhaps an —SH protein or alternatively combination with another cell constituent under the influence of an —SH enzyme. The former possibility is perhaps the most likely in view of the low specificity of the transport system for D-xylose. There is good evidence for combination between sugars and a protein in the case of an enzyme such as hexokinase and it is interesting to note that in the case of brain hexokinase the protein exhibits a lower specificity towards combination with sugars than towards phosphorylation of sugars[10].

Earlier work has shown[3,4] that the activity of monosaccharide transport systems in muscle is enhanced by insulin or by factors which inhibit oxidative phosphorylation. This has led to the conclusions[1]: (1) that the activity of the transport system may be inhibited by reaction with a high-energy phosphate compound or with a compound the intracellular level of which is regulated by high energy phosphate and (2) that insulin activates the transport system by interfering specifically with this reaction sequence. Again it is interesting to note that the activity of a number of enzymes which catalyse reactions involving sugars or derivatives of sugars (that is, proteins combining with these substances) may be regulated by high-energy phosphate or compounds whose intracellular level may be regulated by high-energy phosphate (for example, glucose-6-phosphate). Thus brain hexokinase is inhibited by glucose-6-phosphate[11], the activity of phosphohexokinase may be inhibited by an excess of adenosinetriphosphate[12], and the activities of liver and muscle phosphorylase are capable of regulation by phosphorylation or dephosphorylation of the enzyme[13,14].

We wish to thank Prof. F. G. Young, for his continued interest in these studies. The cost of these investigations was defrayed in part by grants for expenses from the Medical Research Council (to P.J.R.) and from the U.S. Public Health Service (to F.C.B.).

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Occurrence of Astaxanthin in the Flower Petals of *Adonis annua* L.

When the dried petals of *Adonis annua* L. (Ranunculaceæ) (pheasant's eye) are extracted with light petroleum or methanol comparatively large quantities of a deep orange-red carotenoid are obtained. This pigment had an absorption spectrum (in light petroleum, *n*-hexane, carbon disulphate and pyridine) very similar to that of astaxanthin[1]; as the pigment is epiphasic in the 90 per cent aqueous methanol/light petroleum partition test, it would appear to be esterified astaxanthin[2]. The nature of the pigment was confirmed by saponifying the extract. This hydrolyses the esters and converts the liberated astaxanthin into astacin[3] which when the reaction mixture is shaken with ether to extract the unsaponifiable material, collects at the interface as a flocculent orange-red mass. No other known carotenoid behaves in this manner; they are all extractable from a saponification mixture with ether. The flocculent red mass was washed free of alkali and dissolved in dilute hydrochloric acid; as is the case with authentic astacin, the pigment was now extracted from the aqueous phase by ether. The pigment was then purified by chromatography on sugar. Further confirmation of the identification of our pigment with astacin came from various comparisons with authentic astacin (from lobsters); (1) the absorption spectra of the two pigments measured in a number of solvents were identical in position and shape; (2) on co-chromatography on icing sugar using light petroleum containing 2 per cent (v/v) acetone the mixture of the two pigments moved slowly down the column but there was no resolution into two bands; (3) treatment of both pigments with lithium aluminium hydride produces identical pigments (probably 3,4,3′,4′-tetrahydroxy-β-carotene) with absorption spectra indistinguishable from that of β-carotene.

There seems little doubt that the pigment present in the petals of *Adonis annua* L. is an ester of astaxanthin or a very closely related pigment. Up to now astaxanthin, which is widely distributed in invertebrates, has been unequivocally demonstrated in the plant world only in algæ under sterilized conditions[3]. This is the first time that it has been reported in flower petals of a higher plant.

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BIOPHYSICS

Dielectric Studies on Deoxyribonucleic Acid

Deoxyribonucleic acid is a linear polyelectrolyte and a highly asymmetric macromolecule. A specimen, kindly supplied by Dr. K. A. Stacey of the Chester Beatty Research Institute, has been studied in aqueous solution. It was prepared from herring testes by the sodium dodecyl sulphate technique. Dielectric dispersion curves at radio-frequencies have been obtained and the dielectric streaming effect, that is, the variation of the dielectric constant with rate of shear of a solution subjected to a hydrodynamic field, also investigated.

The deoxyribonucleic acid had been purified by high-speed centrifuge and before use was dialysed rapidly for 20 hr. against distilled water at 4°C. so as to be virtually free of salt. This was necessary since difficulties in the method of investigation allow measurements to be made only on solutions with specific conductances less than 40 μmhos cm^{-1}. The dielectric constant was measured over the frequency-range 30 kc./s.–10 Mc./s. using a Schering bridge for low frequencies and a twin-*T* bridge for the high frequencies. The results for three concentrations are shown in Fig. 1. For increasing concentrations the low frequency dielectric increments were 5·0, 7·2 and 9·1 dielectric constant units from which the dielectric increments per gm./l. were calculated to be 54·3, 31·3 and 19·8 respectively. The arrows in Fig. 1 indicate the inflexion points (critical frequencies); these are displaced towards lower frequencies with decreasing concentration. The broken curves have been obtained by use of the theory of Debye[1] in which a single relaxation time is assumed, so that the experimental curves show that a distribution in relaxation times exists. Calculations, using the experimental results and assuming the validity of the Debye concept that the dielectric properties arise from the rotation in an electric field of whole macro-molecules having permanent dipole moments fixed perpendicularly to their long axes, give molecular weights of $0{\cdot}16 \times 10^6$, $0{\cdot}25 \times 10^6$ and $0{\cdot}43 \times 10^6$ for the three concentrations. The weight is higher the lower the concentration. These values are low in comparison with those found by other methods such as light scattering although much higher than the values for thymus deoxyribonucleic acid reported by Jungner, Jungner and Allgen[2] from a similar calculation on results obtained using the Furth ellipsoid method[3,4].

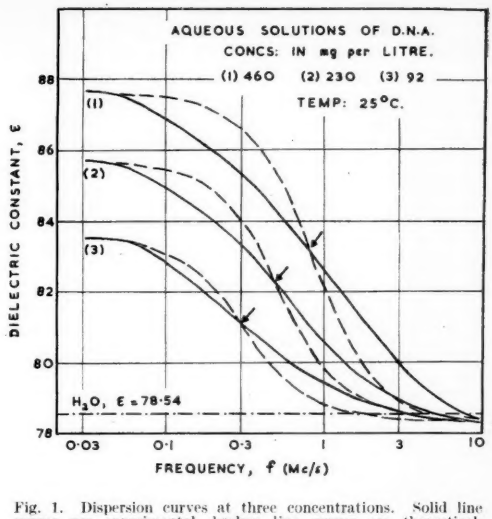

Fig. 1. Dispersion curves at three concentrations. Solid line curves are experimental, broken line curves are theoretical. Arrows indicate critical frequencies

The discrepancy between molecular weight values found by this and other methods and the fact that the molecular weight appears to increase with decreasing concentration cast doubt on the idea that the molecules rotate in their entirety. Further evidence of the inapplicability of Debye's theory in the case of deoxyribonucleic acid is provided by the streaming dielectric effect. When the macromolecules are oriented by a hydrodynamic field all their dipoles should be perpendicular to the direction of flow so that, according to the Debye theory, for an electric field applied perpendicularly to the direction of flow of the dielectric constant should be larger than when random orientation prevails. Experiments made to detect this effect show that the dielectric constant

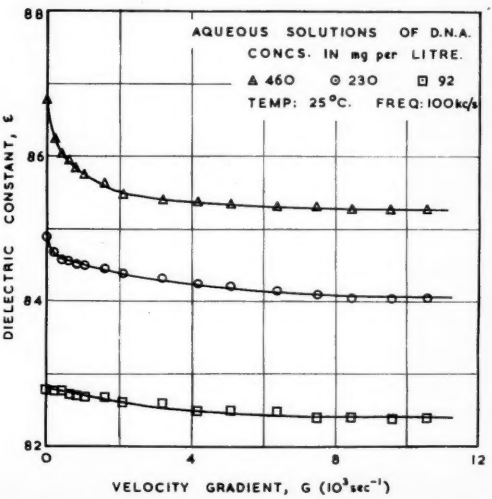

Fig. 2. Variation of dielectric constant with velocity gradient for three concentrations

actually decreases with increasing flow gradient (Fig. 2). The conductivity also decreases. These results are in agreement with those reported by Jacobson[5]. Viscosity measurements made on these solutions showed that their viscosity was a function of shear rate.

In a new series of experiments a solution of deoxyribonucleic acid in 0·2 *M* sodium chloride was heated to 100°C. for 15 min. before dialysis and then examined. It was found that the heating had produced a small decrease in the dielectric increment but no appreciable shift in the dispersion curve. The streaming dielectric effect was present although slightly less pronounced than that in a solution which had not been heated and the viscosity was still a function of shear rate. Another solution of deoxyribonucleic acid was then subjected to the same heat treatment after dialysis. In this case the dielectric increment was considerably reduced and the dispersion curve shifted so that the critical frequency was increased. In addition to these changes the streaming dielectric effect was no longer detectable and the viscous behaviour was apparently Newtonian. If the Debye theory is used for the interpretation of the dispersion results it appears that, whereas the molecular weight of deoxyribonucleic acid was practically unchanged when heated in salt solution, it was reduced by a factor of about eight when heated in the absence of salt. This is in qualitative agreement with the observations of Dekker and Schachman[6] and of Sadron[7].

Measurements, to be reported later, have been made on hyaluronic acid, *Helix pomatia* hæmocyanin and polymethacrylic acid which are closely related physicochemically to deoxyribonucleic acid.

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CHEMISTRY

Effect of Polycyclic Aromatic Hydrocarbons on the Polarographic Maximum of Copper

When a solution of cupric sulphate and sulphuric acid in methanol is polarographed the current-voltage curve shows a pronounced maximum[1], which is sensitive to suppression by a wide variety of substances. Waterman[2] examined the action of polycyclic aromatic hydrocarbons on this maximum, and reported that some carcinogenic hydrocarbons suppressed the maximum to a greater extent than did some non-carcinogenic hydrocarbons. This effect has recently been quoted by Brezina and Zuman[3] as showing an established property of carcinogenic hydrocarbons: but it appears not to have been investigated further. Since Waterman compared 3,4-benzpyrene with pyrene and 1, 2-5, 6-dibenzanthracene with 3-methyl-1, 2-benzanthracene, that is, a five-ring with a four-ring compound in each case, it seemed desirable to carry out experiments with a larger range of hydrocarbons to see whether there is in fact any correlation between carcinogenicity and suppression of the copper maximum.

To 10 ml. of a solution prepared from 0·01 *N* cupric sulphate in redistilled methanol (5 vol.) and 0·1 *N* aqueous sulphuric acid (1 vol.) was added 1 ml. of a 0·004 *M* solution of the hydrocarbon in redistilled methanol as in Waterman's method. Chrysene, 20-methylcholanthrene and the two dibenzanthracenes are however sparingly soluble at this concentration, and the solutions were heated to boiling before withdrawing the 1 ml. After passage of nitrogen, polarograms were recorded photographically using a Cambridge Heyrovsky-pattern polarograph from 0 to about −1·5 V. The cell, with mercury pool anode, was in a thermostat at 25°. The height of the mercury column was 47 cm.; drop time 2·5 sec., drop weight 0·0022 gm., and sensitivity 1/500.

In general it was found that, as the number of aromatic rings in the added hydrocarbon increased, the polarographic maximum of the copper became smaller (Table 1); it was followed by a trough which was just noticeable with anthracene and phenanthrene, but was large with the higher hydrocarbons tested. 1, 2-Benzanthracene suppressed the maximum by about 50 per cent, but a marked additional effect was caused by the aliphatic substituents in 2′, 6-dimethyl- and 9, 10-dimethyl-1, 2-benzanthracenes, and in 20-methylcholanthrene (which can also be regarded as a substituted benzanthracene). There was very little difference, however, between the effects of the non-carcinogenic 2′, 6-dimethylbenzanthracene and the highly carcinogenic 9, 10-isomer.

Table 1. Total Height of Polarographic Maximum of Cupric Sulphate in Methanolic Sulphuric Acid in the Presence of Various Aromatic Hydrocarbons

No. of Aromatic Rings	Hydrocarbon	Height of Maximum (mm.)
—	(none)	50
1	Benzene	47·5
2	Naphthalene	43·5
3	Anthracene	38
	Phenanthrene	33
	1,9-Dimethylphenanthrene	35
4	Pyrene	40
	Chrysene*	29·5
	1,2-Benzanthracene	24
	2′,6-Dimethyl-1,2-benzanthracene	15·5
	9,10-Dimethyl-1,2-benzanthracene†	15
	20-Methylcholanthrene*†	11
5	1,2-3,4-Dibenzanthracene*	14·5
	1,2-5,6-Dibenzanthracene*†	11
	3,4-Benzpyrene†	21

* Owing to their low solubility these hydrocarbons were present in saturated solution.
† Highly carcinogenic hydrocarbons.

Because of their incomplete solubility in this medium the two dibenzanthracenes cannot be too closely compared, but both the carcinogenic 1, 2-5, 6-dibenzanthracene and the virtually non-carcinogenic 1, 2-3, 4-isomer, gave greater depressions than did the carcinogenic 3, 4-benzpyrene which was in complete solution.

Waterman[2] further reported a flattening in the polarogram between −0·3 and −0·4 V. in the case of several carcinogens, but not with 9, 10-dimethyl-1, 2-benzanthracene or with non-carcinogenic hydrocarbons. We sometimes observed this feature with various hydrocarbons, but there is again no correlation with the carcinogenic activity of the hydrocarbon present.

Wawzonek and Laitinen[4] described the direct polarography of a number of aromatic hydrocarbons, which were usually at a concentration of 0·001–0·002 *M* in 75 per cent dioxan containing 0·175 *M* tetrabutylammonium iodide as supporting electrolyte. With the methanolic solvent system and lower hydrocarbons concentrations (0·00036 *M*) used in the present experiments, currents due to the reduction of the hydrocarbons in the absence of copper sulphate were found to be relatively small and have not been taken into account.

Owing to the sparing solubility of some hydrocarbons and the rather poor reproducibility in the medium as used by Waterman, some other possible solvent systems have been briefly examined. When the cupric sulphate, sulphuric acid and methanol were replaced by equivalent amounts of cupric chloride, hydrochloric acid and purified acetone, very pronounced maxima, with only small troughs and very steady diffusion currents, were obtained. Though the copper maximum in this medium was less sensitive to the presence of the hydrocarbons, such depressions as were obtained could still be roughly correlated with the number of aromatic rings, but again not with carcinogenicity. Replacing a portion of the methanol in Waterman's procedure by peroxide-free dioxan permitted complete solution of the less soluble hydrocarbons, but the dioxan itself caused marked suppression of the maximum and the hydrocarbons had little further effect.

It is concluded that the suppressing effect of aromatic hydrocarbons on the polarographic maximum of copper is largely a function of the number of aromatic rings present; the effect can be further enhanced by the presence of some substituents in the rings. There is no evidence of any correlation with carcinogenic activity.

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Szilard-Chalmers Reaction in Lithium Permanganate

The chemical effects following neutron capture by the manganese atom in solid lithium permanganate have been previously investigated[1]. In agreement with the results found by Libby[2] for potassium permanganate, it appears that most of the radioactive manganese in the solid exists in a chemical form, for example, MnO_3^+, which reacts with water to form manganese dioxide and with hydroxyl ion to form permanganate. The active species which exists in the solid undergoes rapid reaction when the solid is dissolved in aqueous solutions. It was felt that additional evidence on the behaviour of the active species might be obtained if non-aqueous solvents were used.

Anhydrous lithium permanganate is soluble in dry pyridine. The salt, after exposure to neutrons from a radium-beryllium source in a paraffin block, was dissolved in this solvent. The percentage of the radioactive manganese which was not removable by shaking the solution with solid MnO_2 was determined as previously described[1]. For aqueous solutions this percentage, termed the retention, corresponds to the percentage of radioactive manganese in the solution which is in the form of permanganate ion. This is not necessarily true for pyridine solutions; hence, this quantity will be termed the apparent retention, the percentage of the activity not removable by MnO_2.

With anhydrous pyridine, the apparent retention for anhydrous lithium permanganate was found to be 95 per cent. However, if the pyridine solution was diluted with 5 per cent of its volume of water before the MnO_2 treatment, the retention gradually dropped, indicating a slow reaction which made the radioactive species more easily removable with MnO_2. With 20 per cent of its volume of water added to the pyridine solution, the apparent retention very rapidly dropped to 8 per cent, the value observed when the irradiated solid was dissolved in pure water. If the pyridine solution was diluted with ten times its own volume of water, the apparent retention rose to about 80 per cent. It is probable that the addition of the large amount of water to the pyridine solution produced a colloidal dispersion of the radioactive manganese dioxide which was not removable by shaking with solid MnO_2 and filtering. Determination of the retention of activity as permanganate ion by precipitation of silver permanganate gave a value of 8 per cent rather than the 80 per cent not removable with MnO_2. Furthermore, the addition of barium hydroxide to the diluted solution followed by neutralization and determination of the retention by silver permanganate precipitation, caused an increase in retention up to 87 per cent. It is known that barium hydroxide promotes an exchange reaction between colloidal manganese dioxide and permanganate ions[1]. The addition of small amounts of water to the pyridine solution thus causes the radioactive species in the solution to form removable manganese dioxide while larger amounts cause its dispersion to form a non-removable colloid.

The active species present in solid neutron irradiated lithium permanganate reacts with aqueous basic solutions to form permanganate ions. Attempts to produce this same reaction in the active species present in the dry pyridine solution were unsuccessful. No non-oxidizable hydroxide base which was soluble in pyridine was available. However, treating the pyridine solution with solid potassium hydroxide, one molar aqueous sodium hydroxide, or a 0·1 molar solution of potassium hydroxide in a 50-50 mixture of pyridine and tertiary butyl alcohol gave no evidence of conversion of the active species to permanganate ion. Subsequent addition of small amounts of water could reduce the apparent retention to 8 per cent as before. Evidence was not obtained that the species present in pyridine solution underwent the same type of reaction with hydroxide bases as did the active species present in the irradiated solid.

There is evidence that at least part of the radioactive species present in the dry pyridine solution of the irradiated solid can undergo some reactions with added MnO_2. If the pyridine solution is treated with MnO_2, filtered, and 20 per cent of its volume of water added to the filtrate, a subsequent treatment of the

filtrate with MnO_2 yields an apparent retention of 80 per cent instead of the value of 8 per cent which is observed if the first MnO_2 treatment is omitted. The same result is obtained following the addition of Mn_2O_3 or $Mn(ClO_4)_2$. In the absence of water these substances thus cause the conversion of most of the active species to a form which is not made removable by subsequent addition of water and shaking with MnO_2. The high apparent retention of 80 per cent is not due to conversion of the active species to permanganate since the retention as permanganate, determined after dilution and precipitation of silver permanganate, is 8 per cent. The increase in permanganate retention which was observed when the diluted solution was treated with barium hydroxide suggests that the remaining 72 per cent of the activity was present as colloidal MnO_2, not easily removable by shaking with solid MnO_2.

In conclusion, it has not been demonstrated that the active species in dry pyridine solution is similar in its reactions to the active species in the solid. After addition of water to the pyridine solution, the active species appears to be present as manganese dioxide which, under some conditions, forms a colloidal dispersion in the pyridine-water solution.

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N-Methylation of Methyl 2-Acetamido-2-Deoxy-α-D-Glucopyranoside

Type XIV *Pneumococcus* polysaccharide, which contains *N*-acetyl glucosamine, glucose and galactose residues[1], has been methylated using dimethyl sulphate and sodium hydroxide (Haworth method[2]) to yield a methyl ether (I) which on hydrolysis gave 3-*O*-methyl D-glucosamine as the only methylated hexosamine.

By contrast, hydrolysis of a methyl ether (II) obtained from Type XIV *Pneumococcus* polysaccharide using sodium and methyl iodide in liquid ammonia (Freudenberg method[3]) gave as the only methylated amino-sugar a compound having the properties expected of 3-*O*-methyl-2-methylamino-2-deoxy-D-glucose. Moreover, the infra-red spectrum of II differed from that of I in that only the latter showed absorption at 1540 cm.$^{-1}$ attributable to the N-H deformation of an acetamido group[4].

These findings, together with the lack of mobility of II in acetate buffer *p*H 5 and its failure to give a colour with ninhydrin indicated that II contained *N*-methyl acetamido groups. This novel feature of the Freudenberg methylation procedure[3] has now been confirmed using methyl 2-acetamido-2-deoxy-α-D-glucopyranoside as a model compound.

D-Glucosamine hydrochloride (5 gm.) in 10 per cent aqueous methanol (110 ml.) was stirred for 90 min. at 0–5° with 'Amberlite *IRA*-400' (CO_3^{--}, 140 ml.) and acetic anhydride (3 ml.)[5]. The filtrate was passed through a column of 'Amberlite *IR*-120' (H^+, 25 ml.) and concentrated. The product (4·47 gm.) was refluxed with dry methanol (150 ml.) and 'Amberlite *IR*-120' (H^+ form dried over phosphorus pentoxide at 80°, 4·47 gm.) for 3 hr.[6]. Concentration of the filtrate yielded methyl-2-acetamido-2-deoxy-α-D-glucoside (3·82 gm.; m.p. 184°). This model compound was submitted to one methylation[3] involving four alternate additions of sodium (total, 12 gm.) and methyl iodide (total, 36 ml.) in liquid ammonia (300 ml.). After removal of ammonia, an aqueous solution of the product was extracted with water-washed chloroform and the extracts concentrated to a yellow syrup. Most of the syrup (2·2 gm.) distilled at 128°/0·11 mm., had $n^{18.5}$ 1·4660 and showed $[\alpha]_D^{21}$ +93·5 (*c.*, 3·3 in chloroform). Its infra-red spectrum included strong absorption at 1640 cm.$^{-1}$ (C=O stretching vibration) but none at 1540 cm.$^{-1}$ (absence of N-H deformation). Analysis indicated that it was a crude specimen of methyl-3:4:6-tri-*O*-methyl-2-*N*-methylacetamido-2-deoxy-α-D-glucoside. Found: OMe, 41·2; CH_3CO, 16·2 per cent. $C_{13}H_{25}O_6N$ requires OMe, 42·5; CH_3CO, 14·8 per cent. To confirm this a portion (500 mgm.) was hydrolysed with 3N-hydrochloric acid (3 ml.) at 100° for 6 hr. and the product crystallized. After recrystallization from dry ethanol it decomposed in the range 170–200°, and showed $[\alpha]_D^{20}$ +105° equil. (*c.*, 0·12 in water) as expected for 3:4:6-tri-*O*-methyl-2-methylamino-2-deoxy-D-glucose hydrochloride.

Fried and Stavely[7] quote a decomposition range of 160–210°, $[\alpha]_D$-108° for the L-isomer. On paper chromatograms this hydrochloride and 3:4:6-tri-*O*-methyl-2-amino-2-deoxy-D-glucose hydrochloride had respectively R_F values of 0·65 and 0·56 in butanol-ethanol-water (4:1:5), 0·51 and 0·46 in butanol-acetic acid-water (4:1:5) and of 0·60 and 0·53 in butanol-ethanol-water-ammonia (4:1:4·9:0·1). Part of the hydrochloride (284 mgm.) was dissolved in water (2 ml.) and passed through a pad of charcoal and silver oxide onto freshly precipitated yellow mercuric oxide (5 gm.). The mixture was heated on a boiling water bath for 15 min. and then refluxed for 15 min. On concentration and addition of alcohol, crystals of 3:4:6-tri-*O*-methyl-2-methylamino-2-deoxy-D-gluconic acid were obtained, m.p. 204° (Fried and Walz[8] quote m.p. 206–207°).

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The Unit Cell of Mercuripapain Crystals

Mercuripapain was prepared and crystallized for the first time by Kimmel and Smith[1]. It contains one atom of mercury for two molecules of papain, but the nature of the mercury-protein complex is not exactly known. Papain is a proteolytic enzyme and has a molecular weight of 20,500 (ref. 2).

In order to find out if a structure determination of mercuripapain crystals could be successful, we determined the dimensions and the symmetry of the unit cell. Our preliminary results are reported here.

Papain was obtained from the Worthington Biochemical Corporation and crystallized with mercuric chloride, as described by Kimmel and Smith, in the cold from 70 per cent aqueous ethanol (by volume). Crystals grew in a few weeks. They are lath-like plates, some tenths of a millimetre long and across, but only some hundreths of a millimetre thick. X-ray diffraction pictures were taken at room temperature. The crystals were mounted in thin-walled glass capillaries; they were in equilibrium with their mother liquor. A precession camera and nickel-filtered copper radiation from an ordinary sealed-off X-ray tube were used. For a 9° precession angle an exposure time of 20 hr. was required. Reflexions were observed out to at least 3 A. The crystals appeared to be monoclinic, space group $P2$; $a = 56{\cdot}0 \pm 0{\cdot}2$ A.; $b = 49{\cdot}5 \pm 0{\cdot}2$ A.; $c = 41{\cdot}8 \pm 0{\cdot}2$ A.; $\beta = 96{\cdot}2 \pm 0{\cdot}2°$. Air-dried mercuripapain crystals showed scarcely any X-ray reflexion. Therefore no data could be obtained for the dry unit cell.

The density of air-dried crystals is 1·291 gm./ml. and of crystals dried over calcium chloride at low pressure 1·303 gm./ml. These values were obtained with the gradient column technique. The column components were chloro- and bromo-benzene. This method could not be adopted for the wet crystals because they contain ethanol and this is soluble in the liquid of the gradient column. Suitable column components for this case are not easy to find. However, for calculating the number of papain molecules in the unit cell we need the density of the wet crystals. This was determined with a small pycnometer. The weight of the pycnometer containing wet crystals was determined before and after it was filled with mother liquor. From the known density of the mother liquor, the density of the crystals was calculated to be $1{\cdot}15 \pm 0{\cdot}02$ gm./ml.

The solvent content of the wet crystals was obtained by weighing them in the wet and the dry state. In the wet state they were freed from adhering mother liquor with filter paper. To prevent the loss of liquid of crystallization, this was carried out in an atmosphere saturated with the vapour of 70 per cent ethanol. The dry state was reached at low air pressure over calcium chloride. The wet crystals contain 45 ± 2 weight per cent of liquid of crystallization.

We are now able to calculate the protein content of the unit cell: there are two papain molecules with molecular weight $22{,}000 \pm 1{,}500$. This value is in good approximation to the value of 20,500 given by Smith and Kimmel as estimated by a variety of methods. The two papain molecules in the unit cell are related by the twofold axis.

Though the analytical results of Kimmel and Smith indicate a combining ratio of 1 mole of mercury to 2 moles of papain, a dimeric complex was not found by these workers; but from electrophoretic and ultra centrifuge experiments they concluded that mercuripapain contains a mixture of monomers and a heavy complex with approximately six times the size of the monomer[3]. They emphasized that this heavy complex would be the least soluble type under the conditions of crystallization from 70 per cent ethanol. Therefore it was anticipated that the unit cell would contain at least six molecules of papain. Actually there are only two, so the heavy complex is not incorporated in the crystal structure.

Our results have encouraged us to continue the work on the structure of mercuripapain and papain.

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Structure of Cellobiose

The structure of cellobiose is of interest in connexion with related structures of cellulose, regenerated cellulose, and other degradation products. We have obtained a three-dimensional structure of cellobiose by single-crystal X-ray diffraction methods.

The crystals are monoclinic with two molecules in a unit cell with parameters: $a = 10{\cdot}94$, $b = 13{\cdot}05$, $c = 5{\cdot}11$ A. and $\beta = 90{\cdot}0°$ in the space group $P2_1$ (ref. 1). The atomic arrangement (Fig. 1) was obtained by quantitative analysis of a modification of the three-dimensional Patterson function. This modification made use of the new function:

$$P'(u, v, w) = \int \nabla\rho(x, y, z) \cdot \nabla\rho(x+u, y+v, z+w) \mathrm{d}V$$

$$\propto \sum_{hkl} \frac{\sin^2\theta}{\lambda^2} |F_{hkl}|^2 \exp[2\pi i(hu + kv + lw)]$$

computed from $|F_{hkl}|^2$ values which had already been sharpened by the usual methods. We found it advisable to add a small contribution of the usual Patterson function in order to decrease negative regions around the peaks. Thus, this new procedure emphasizes the gradient of the electron-density function, and was of considerable importance in resolving closely neighbouring peaks.

The resulting series was used in a number of three-dimensional superpositions by procedures which made it possible to calculate the 'Buerger minimum function'[2] on the Remington Rand 1103 computer. The structure was refined by least-squares methods almost completely in the *c*-axis projection (Fig. 1)

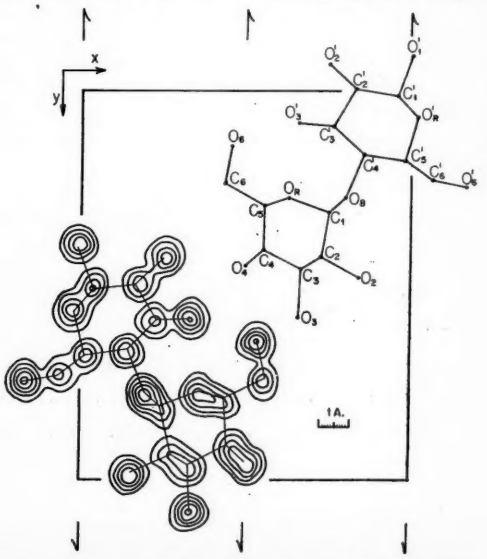

Fig. 1. Projection of the cellobiose structure on to the (001) plane. Contours are drawn in arbitrary units

and, so far, by five cycles of three-dimensional least-squares refinement. At this stage of refinement, $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|=0{\cdot}20$, and[3] $r=\Sigma w(|F_o|^2-|F_c|^2)^2/\Sigma w|F_o|^4=0{\cdot}07$ for all data, and $R=0{\cdot}13$ for the $hk0$ data. These values indicate that the structure is basically correct. In addition, shifts of distance and temperature parameters in the least-squares analysis are small, comparable with present standard deviations. Present C—C distances are $1{\cdot}53\pm0{\cdot}11$ A., and C—O distances are $1{\cdot}49\pm0{\cdot}07$ A. Hydrogen bonding will be shown in detail in our full publication. The relative configuration of the two rings can be derived most easily from the co-ordinates shown in Fig. 1. However, the relative orientation of the two rings in the molecule (Fig. 1) is sufficiently different from the one previously assumed[4] to suggest that the problem of the structure of regenerated cellulose may need re-examination.

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ANIMAL PHYSIOLOGY

The C_{17}-OH Group of Œstradiol: Relationships between its Anti-Atherogenic and Endocrine Actions

Although œstrogens prevent coronary atherosclerosis in experimental animals[1], related mechanisms of action have not been elucidated, nor is it universally accepted that prevention of atherosclerosis and endocrine activity are univocally related. Advantage was taken of the fact that two isomers of œstradiol, namely, α- and β-œstradiol, have very different endocrine activity, whereas the anti-atherogenic effects have not been so far determined (α-œstradiol, △-1,3,5 (10) œstratriene, 3β, 17α diol (m.p. 197°C.) generously provided by Laboratorios Dr. Gador, Buenos Aires, through Dr. Estoban Montuori, who also kindly prepared the pellets of approximate weight 25 mgm. each).

Six-weeks-old White Leghorn cockerels were housed in large pens and fed commercial chick starter mash for one week. They were then divided into several groups: group 1 (intact controls), consisted of 12 animals on commercial mash; group 2 (cholesterol controls), 13 birds received mash supplemented with 2 per cent cholesterol; group 3 (experimental β), 11 chickens received the same diet plus subcutaneous pellet implantation of β-œstradiol 1, 20 and 45 days after the beginning of cholesterol feeding; group 4 (experimental α) was similar to group 3, but pellets containing α-œstradiol were implanted in 12 animals. This timing ensures maximal absorption of the drug[2,3]; group 5 (sham-operated controls) 6 birds received the cholesterol supplemented diet and were sham-operated on the same days as those chickens from groups 3 and 4. Since results obtained were similar, groups 2 and 5 are reported together.

The birds were killed after 8 weeks and the following were examined: (1) plasma cholesterol-levels[3]; (2) plasma and aortic alkaline phosphatase activity[4,5]; (3) aortic macroscopic and coronary microscopic atherosclerosis arbitrarily graded from 0 to 3 (slightly modified as described in ref. 5); (4) weight and microscopic anatomy of endocrines. Determinations were performed 'blindly' and compared by Students's t test according to Fisher's tables (ref. 6).

Results shown in Table 1 demonstrate that both isomers increased body-weight and induced testicular atrophy, while increasing, at the same time, alkaline phosphatase activity in plasma and in the aorta. Only the β-isomer was able simultaneously to elevate cholesterolæmia and to prevent coronary atherosclerosis.

The effects of both drugs on the various activities examined indicate, as a consequence, a clear-cut difference: while the β-position of the C_{17}-OH group was not essential for inducing testicular atrophy or for increasing alkaline phosphatase in the aorta and in the blood, only β-œstradiol was able to increase blood cholesterol-levels and to prevent coronary atherosclerosis. It is evident, therefore, that although the endocrine and vascular effects of natural œstrogens are generally associated, even in the human being they can also be separated as has already been shown in rats and in rabbits[7]. Furthermore, it would seem from the present experiment, that in birds, modification of cholesterol metabolism and prevention of coronary atherosclerosis is related to the β-position of the C_{17}-OH group in the œstrane molecule. Further results will be published in more detail elsewhere.

Table 1. Results obtained in cholesterol-fed chickens implanted with œstradiol isomers (mean ± standard deviation of the mean)

Group	No. of animals	Body weight (gm.)	Testicles (mgm./kgm.)	Plasma cholesterol (mgm./100 ml.)	Plasma alkaline phosphatase (micromoles of phenol liberated/ml.)	Aorta alkaline phosphatase (micromoles of phenol liberated/mgm. of wet tissue)	Aortic macroscopic atherosclerosis (number of birds)	Coronary microscopic atherosclerosis (grade)
(1) Intact control	11	817±115	618·5±173·2	81±6·59	62±4·60	0·094±0·0068	0	0
(2) Cholesterol-fed	15	815±64·3	400·7±61·4	154±14·14	72·5±4·81	0·108±0·0070	0	8·25±2·02
(3) Cholesterol-fed,17 − β œstradiol	8	1043±56·21	212·8±11·7	404±25·49	248·7±23·52	0·131±0·010	2	0·5±0·14
(4) Cholesterol-fed,17 −α œstradiol	12	1023±50·51	209·8±27·5	187±20·22	243±45·05	0·158±0·023	0	9·70±2·54
"t" 1 vs. 2			1·930 $P>0{\cdot}05$	4·709 $P<0{\cdot}001$	1·583 $P<0{\cdot}20$	1·443 $P<0{\cdot}20$		
"t" 2 vs. 3		3·904 $P<0{\cdot}001$	3·090 $P<0{\cdot}01$	8·620 $P<0{\cdot}001$	7·341 $P<0{\cdot}001$	2·300 $P<0{\cdot}05$		3·836 $P<0{\cdot}001$
"t" 2 vs. 4		3·918 $P<0{\cdot}001$	2·849 $P<0{\cdot}001$	1·341 $P>0{\cdot}10$	3·814 $P<0{\cdot}01$	2·096 $P<0{\cdot}05$		0·484 $P>0{\cdot}6$
"t" 3 vs. 4		3·904 $P<0{\cdot}001$	0·101 $P<0{\cdot}001$	6·867 $P<0{\cdot}001$	0·111 $P>0{\cdot}90$	1·125 $P<0{\cdot}30$		

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Relation of Ethanol Metabolism to Several Factors in the Rat

The most important site for the oxidation of alcohol is the liver. Various experimental approaches, for example, the artificial perfusion of surviving liver with blood containing alcohol, oxidation of alcohol by liver slices and liver brei, diminished alcohol oxidation following either hepatectomy or damage to the liver, and the demonstration that the eviscerated animal metabolizes alcohol very slowly, have all indicated that the liver is of major importance in the metabolism of ethanol[1].

Eggleton[2], in an extensive study in cats, attempted to find one or more of the important factors responsible for the varying rates of alcohol metabolism between individuals of the same species. It was found that the rate of ethanol metabolism correlated more nearly with liver-weight than with body-weight or surface area. In the present study, instead of using mongrel animals, we have attempted to obtain consistent results by using a pure strain of rat, for example, Sprague-Dawley females. Additionally, an attempt was made to determine the alcohol-oxidizing capacity of liver by modifying the methods of Bücher and Redetski[3] and Marshall and Fritz[4] for use in the coenzometer (Macalaster Bicknell Co.). It is evident in Table 1, summarizing the findings in 17 female rats, that the results obtained with this modified method fail to correlate significantly with the rate of alcohol metabolism where the latter is expressed in terms of the amount of alcohol disappearing from the blood[5]. The finding of Eggleton[2] in the cat that the rate of metabolism is more closely related to liver-weight than to body-weight is confirmed in the rat.

Table 1. Relation between rate of ethanol metabolism (*Disappearing Ethanol*) and several variables

	Mean ± *S.D.**	Cor. Coef.
Ethanol metabolism (Ethanol disappearing) (mgm./kgm./hr.)	249·0±57·9	—
Liver oxid. capacity, wet basis (mgm./gm. liver/hr.)	2·74±0·40	0·291*
Liver oxid. capacity, N_2 basis (mgm./gm. N_2/hr.)	103·6±14·0	0·382*
Liver-weight (gm.)	7·5±0·6	0·485*
Body-weight (gm.)	327·0±29·4	0·082*
Liver-weight : body-weight (per cent)	2·31±0·27	—

* Mean ± Standard Deviation of Mean; *$P>0·05$.

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Effect of Tranquilizing Drugs on Survival from Experimental Diphtheric Intoxication

The exotoxin of virulent diphtheria bacilli represents an extreme example of a potent biochemical stressor in susceptible mammalian species. Giroud *et al.*[1] have shown that in animals intoxicated with a lethal dose of diphtheria toxin, the hormone content of the adrenal cortex is diminished by as much as two thirds. Attempts to influence the course of diphtheria toxæmia through the use of cortical hormones or adrenocorticotrophic hormone have met with no success[2,3,4], although a decrease in adrenal hæmorrhages has been noted[5].

In view of the potent pharmacological activity of the tranquilizing drugs in stress states, it seemed of interest to determine the effects of such agents upon experimental diphtheric stress. Since mice and rats are refractory to the toxin, large male guinea pigs were employed.

Using groups of eight or more animals paired by weight, age and source, four major tranquilizers were evaluated. These drugs, representing each of the four chemical classes of tranquilizing drugs were meprobamate, chlorpromazine, reserpine and hydroxyzine. Diphtheria toxin, partially purified from broth, with an LD_{50} of approximately 3×10^5/ml., was used as the challenge. A dose of 0·1 ml. undiluted, sterile toxin was injected intraperitoneally, and

Table 1. Survival Time of Guinea Pigs Treated Prior to Challenge with Diphtheria Toxin

Survival time as % of Control ± Standard Error	Significance of difference from Control	Experimental treatment (single injection, i.p.) (mgm./kgm.)	Survival time* of Controls ± Standard Error	Degrees of Freedom
		(A) TRANQUILIZER TREATMENT		
140±7	$P<0·001$	Meprobamate 400	100±5	14
115±4	$P<0·02$	Meprobamate 300	100±3	15
111±14†	$P>0·4$	Meprobamate 500	100±4	18
110±4	$P>0·1$	Reserpine 1·0	100±5	14
103±2	$P>0·4$	Meprobamate 200	100±3	15
100±4	$P>0·9$	Chlorpromazine 25	100±3	14
96±2	$P>0·3$	Hydroxyzine 2·5	100±3	14
94±6	$P>0·3$	Hydroxyzine 25	100±3	14
92±10	$P>0·4$	Reserpine 0·1	100±5	14
89±3	$P>0·05$	Reserpine 1·4	100±3	14
87±3	$P<0·01$	Reserpine 2·5	100±3	18
60±8	$P<0·001$	Chlorpromazine 200	100±3	14
		(B) MISCELLANEOUS TREATMENT		
106±3	$P>0·1$	Chloral Hydrate 400	100±3	14
101±4	$P>0·8$	Tubocurarine 0·25	100±3	13
97±3	$P>0·4$	Pentobarbital 20	100±3	14
76±17**	$P>0·1$	Hexobarbital 100	100±3	13

* The overall mean survival time for all controls was 13 hr. and the average standard error for all control groups was 25 min. (3 per cent).
† The large standard error associated with the dose of 500 mgm./kgm. meprobamate can be attributed to 2 early deaths from the drug (which is close to the LD_{50} at this dose). If these two deaths are removed from the calculations, survival time for this group was 131±6 per cent of its control, which with 16 degrees of freedom is a significant increase, $P<0·001$.
** The large standard error is attributed to the fact that 3 animals died within three hours when given 100 mgm./kgm. hexobarbital. If the calculations are made without these 3 animals, the survival time of the group is 110±6 per cent of its control. With 10 degrees of freedom, this increase is not significant, $P>0·1$.

untreated animals always died in approximately 13 hr., in keeping with the established fact that regardless of the dose of diphtheria toxin employed, experimental animals never die in less than 10 hr.[6] Tranquilizers were diluted in sterile saline except for meprobamate which was diluted with 5 per cent acacia solution. The drugs were injected intraperitoneally approximately one hour prior to the toxin challenge, and all solvents were shown to be without effect in altering the lethality of the toxin.

Table 1 shows the effects of the four test agents at various dose-levels in descending order of influence on survival time compared to the corresponding control group. From the data presented, it appears that these four drugs differ markedly in ability to influence the course of diphtheric stress. Meprobamate at all doses tested above 200 mgm./kgm. significantly enhanced survival time in all trials, while large doses of chlorpromazine significantly enhanced the lethality of the toxin. This response to chlorpromazine corresponds to previous findings of increased susceptibility to infection with large doses of the drug[7]. Reserpine apparently showed a critical level at approximately 1 mgm./kgm. In doses above this, the drug reduced survival time significantly, while doses below this did not. Neither small nor large doses of hydroxyzine affected the survival time in our trials.

To preclude the possibility that alterations in survival time were caused by the sedative action of the tranquilizing agents, chloral hydrate, pentobarbital and hexobarbital were administered to groups of animals prior to diphtheria toxin. In the dosages employed, the animals slept longer than in any of the tranquilizer runs. As can be seen from Table 1, none of these classical hypnotics significantly influenced the survival time. The possibility that the muscle relaxant properties of several of the tranquilizers might be associated with the alterations in survival time was investigated using tubocurarine in a dose of 0·25 mgm./kgm. administered immediately prior to the injection of toxin. This produced a non-sedative, muscle-relaxing state in the animals for some time, but no influence on the survival time was noted.

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Distribution of Promethium Plasma Fractions in Rats

Differences in relative particle-sizes of metals chelated *in vitro* and injected into animals have been related to their selective deposition in tissues[1]. The possibility that injected inorganic salts of metals may also combine with normal plasma constituents to influence their subsequent distribution among animal tissues might be surmised from many physico-chemical considerations. The present investigation presents preliminary results which indicate that selective distribution in tissues of radioactive promethium (^{147}Pm) may be related to combinations with plasma fractions.

A young adult female Wistar rat (247 gm.) was injected intravenously with 900 μc. promethium chloride (^{147}Pm Cl_3), *p*H 1·7. Three minutes later, approximately 5 ml. of blood was removed from the animal and centrifuged. Aliquots of the plasma were subjected to two procedures: (1) dialysis against isotonic saline for 48 hr. at 1°C.; the measured ratio of concentration of radioisotope in the dialysate, *D*, to that in the nondialysable fraction, *N*, was 1:19; (2) gamma globulin and albumin fractions were isolated from undialysed plasma by a buffered alcohol precipitation procedure[2]. The purity of these protein fractions labelled with promethium-147 was confirmed by paper electrochromatography following 48 hr. dialysis.

Aliquots of the protein isolates and the two whole plasma fractions, diluted with isotonic saline, were injected intravenously into 18 young adult female rats.

Concentrations of promethium-147 in serial tail-blood samples were measured for 1 hr. following injection, but did not vary much between groups. At the end of this time, the animals were killed and the percentage distribution of promethium-147 in several tissues and accumulated bladder urine was measured. The results are shown in Table 1.

The promethium-147 administered with the *D* fraction had concentrated in tissues and plasma more than when injected in the *N* fraction. These statistically significant differences ($P < 0{\cdot}01$) indicate, in view of the low *D*/*N* ratio (1:19), great selectivity in tissue concentration of promethium-147, possibly related more to comparative dialysability (diffusibility) of the fractions, than to the relative amounts present. There is also the possibility that 'chemical interactions' between constituents which comprise the fractions and the tissues may influence the concentration of promethium-147.

In contrast, the low concentrations of promethium-147 in several tissues after injection of the *N* fraction may accord with distribution in tissue spaces rather than specific combination with tissue, although on the basis of the available data it is not possible to ascertain accurately the relative importance of these possibilities following injection of either *D* or *N* fractions. Analogous phenomena may account to some extent for the disparity between the tissue

Table 1. Distribution and Excretion of Promethium-labelled Plasma Fractions
(Mean ± standard error)

Tissue Fraction	Liver	Kidney	Spleen	Femur*	Plasma Per cent promethium-147	Urine Per cent promethium-147
	Per cent promethium-147/gm.					
Dialysate, *D*	5·45 ± 0·84†	1·25 ± 0·32†	8·01 ± 1·79†	1·39 ± 0·53†	0·71 ± 0·09†	0·41 ± 0·16
Nondialysed, *N*	1·43 ± 0·12	0·46 ± 0·10	0·35 ± 0·12	0·10 ± 0·007	0·08 ± 0·005	0·25 ± 0·13
Albumins, *A*	6·05 ± 2·55	2·57 ± 0·79	3·95 ± 0·83	1·46 ± 0·71	0·39 ± 0·07	5·79 ± 2·22
γ-Globulin, *G*	2·53 ± 1·01	0·77 ± 0·44	2·91 ± 1·58	1·30 ± 0·73	0·04 ± 0·007	0·90 ± 0·14
Ratio, *A*/*G***	2·4	3·3	1·4	1·1	9·8	6·4

* Femur weights corrected for measured mean value of 25 per cent for marrow weight.
† Significant difference from non-dialysable fraction at 1 per cent level.
** Normal ratio, albumin/γ-globulin, = 12·9 (ref. 3).

concentrations of promethium-147 observed after the two labelled albumin and gamma globulin isolates were administered and their normal plasma ratio. Further study is required to establish these possibilities.

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The Anterior Pituitary-like Activity of Rumen Contents

IT is well established that alimentary microflora can synthesize vitamins which become available for the use of animals. The tremendous number of these Protozoa suggests that they may be of use to ruminants. Most investigations have been directed hitherto towards the role taken by the microflora and microfauna in digestion and vitamin synthesis, but there has been no definite information regarding the importance of the part played by the Protozoa in ruminants[1]. It has been reported that œstrogen and thyroxine exert an inhibitory effect on the growth of some bacteria[2]. Others have reported that the gonadotrophins of pregnant women's urine cause marked growth stimulation of Protozoa[3]. As there was a possibility that growth and reproduction of these micro-organisms are associated with some hormonal activity, it was decided to find out if rumen contents, Protozoa and bacteria possess hormonal activities.

The thyrotrophic, gonadotrophic and adrenocorticotrophic hormone activity of rumen fluids of cows, buffaloes and sheep were tested using the one-day-old male chick method of assay[4]. The thyroids, testes and adrenals of young chicks are sensitive to these specific trophic hormones and are the only suitable intact animals for the assay of such hormones[5]. For extraction of these hormones the gauze-filtered rumen fluids obtained from five animals of each species were centrifuged at 3,000 rev./min. for 15 min. and 1000 ml. of the supernatant fluid was withdrawn and to it an equal volume of acetone was added together with a few crystals of sodium chloride. The sediment which resulted from the addition of acetone was obtained by centrifugation at 2,000 rev./min. for 5 min. and was then re-dissolved in saline. The extract was purified by repeating this procedure twice while gradually decreasing the volume of the solvent (saline). Amounts of extracts equivalent to 10, 20 and 50 ml. of rumen fluids were then injected into male frogs (*Bufo regularis*). They showed no evidence of the presence of gametokinetic activity[6].

The chicks were divided into groups of 15 each. Extracts equal to the original volume of the rumen fluids used (Table 1) were divided into 4 daily injections and the chicks were killed on the fifth day. As will be seen from the results in Table 1, the rumen contents of cows, buffaloes and sheep possess an anterior pituitary-like activity, inducing a statistically significant increase (using the '*t*' test) in the weights of chick testes, adrenals and thyroids, when compared with those of the controls injected with saline.

The next step was to attempt to isolate such hormones from rumen Protozoa and Bacteria. Rumen Protozoa (*Diplodinium ecaudatum*) were grown on a medium of baked lettuce extract. After 48 hr., when the division of Protozoa reached a maximum, they were counted using a hæmocytometer. The hormones were extracted by the addition of equal volumes of acetone in a manner similar to that already mentioned. As to Bacteria, *Lactobacillus acidophlus*, a predominant organism present in the rumen of adult bovine fed hay and concentrates[7], was isolated from the rumen contents of cows. It was grown on agar and collected with sterile saline, centrifuged and washed twice with saline, and the collected organisms were weighed, dried with acetone, re-dissolved in saline and their hormone contents extracted with acetone as before. *Escherichia coli* were also grown on agar and the crop was cultivated with the aid of sterile saline solution and the hormones extracted with acetone.

The hormonal activity of Protozoa and Bacteria were tested using the one-day-old chick assay method[2], but since the gonadotrophic activity of the Protozoa by this method did not give significant results, further investigations were made by injecting the extract into male and female immature mice[5]. The results obtained indicate clearly that rumen Protozoa, *L. acidophilus* and *E. coli* extracts have hormonal activities of gonadotrophic, adrenocorticotrophic and thyrotrophic nature. Injection of protozoal extracts caused a significant increase in the weight of testes and seminal vesicles of male mice, with the exception

Table 1. INFLUENCE OF RUMEN FLUIDS, PROTOZOA AND BACTERIAL EXTRACTS ON TESTES, ADRENAL AND THYROID WEIGHTS OF CHICKS

Test material	Total dose	Organ weights (mgm./100 gm. body-weight) Testes	Adrenal	Thyroid
Rumen Fluid:				
Cow	20 ml.	30·44† ±4·64	35·96 ±3·04	14·68† ±1·00
	30 ml.	30·31† ±4·72	36·82 ±2·85	14·74† ±0·79
Control	—	14·73 ±1·14	34·83 ±5·57	10·67 ±0·57
Sheep	10 ml.	31·24† ±4·12	48·06 ±2·86	13·76* ±1·00
Buffalo	10 ml.	34·06† ±2·45	40·30 ±4·17	12·88* ±1·00
Control	—	20·14 ±1·87	38·44 ±5·90	10·84 ±1·00
Lactobacillus acidophilus	0·04 gm.	31·16† ±2·85	42·08† ±3·03	16·20† ±2·40
	0·08 gm.	23·26 ±2·00	42·80† ±2·19	18·10† ±2·50
Control	—	18·89 ±1·36	28·62 ±2·93	8·30 ±0·95
E. coli extract	0·1 gm.	33·18† ±5·74	45·67† ±2·05	14·21† ±1·44
	0·2 gm.	35·20† ±3·77	50·32† ±4·30	14·74† ±2·00
Protozoa	90 million cells	21·38 ±2·00	36·62* ±2·74	11·98* ±0·93
Control	—	22·68 ±2·05	28·13 ±2·05	9·04 ±0·91

* "*t*" test, significant at 5 per cent level.
† "*t*" test, significant at 1 per cent level.

Table 2. INFLUENCE OF PROTOZOAL EXTRACT ON REPRODUCTIVE ORGANS OF MALE AND FEMALE MICE

Treatment	Male mice: Testes weight (mgm./100 gm.)	Male mice: Seminal vesicle (mgm./100 gm.)	Female mice: Ovary weight (mgm./100 gm.)	Female mice: Uterus weight (mgm./100 gm.)
Control	739·5 ±54·7	173·0 ±29·7	15·60 ±1·04	166·5 ±15·2
20 million cells	952·1† ±23·4	246·3 ±38·5	21·57† ±0·88	157·0 ±7·35
40 million cells	893·9† ±31·7	313·5† ±40·1	19·04* ±0·55	110·0 ±9·79
80 million cells	535·3† ±77·1	62·9† ±14·3	—	—

* Significant at 5 per cent level.
† Significant at 1 per cent level.

of the large dose (Table 2) which caused pronounced atrophy of both testes and seminal vesicles. This is probably due to the formation of anti-gonadotrophins. In the case of female mice, the protozoal extract induced a significant growth of the ovaries but had no effect on the uterus.

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A Heat-Loss Mechanism involving the Habenular, Interpeduncular and Dorsal Tegmental Nuclei

A region of the brain sensitive to temperature has been described as between the optic chiasma and the anterior commissure in the cat[1]. Direct heating of this region with diathermy was shown to induce panting. In the goat, panting was found[2] to continue for 1–2 min. after the end of 5 min. of electrical stimulation in the corresponding region of the brain, and to be effective in lowering the temperature of the animal. Moderate heating of this region in the cat and the rabbit was also found[3] to reduce gamma motor activity. The present experiments serve to identify an efferent pathway from this temperature-sensitive region.

In rabbits anæsthetized with urethane, ether or pentobarbital electrical stimulation in a wide region between the optic chiasma and the anterior commissure can be shown to induce both panting and an inhibition of somatic reflexes such as the knee jerk. If stimulation at 25–50 pulses per sec. is continued for more than 10 sec. the panting and inhibition of the knee jerk reflex continue for 1–3 min. after the end of stimulation. This syndrome of panting and inhibition of the knee jerk reflex ending simultaneously and often fairly suddenly many seconds after the end of stimulation can also be elicited by electrical stimulation of the habenular (Fig. 1), interpeduncular or dorsal tegmental nuclei. These nuclei are interconnected by well-known tracts and form a system of unknown function. The typical syndrome is not elicited by stimulation in other regions including the hypothalamus, the hippocampus and the medial or lateral septal nucleus.

Electrical stimulation in the region sensitive to temperature evokes an electrical response in the ipsilateral habenular nucleus with a latency to first inflexion of 3 msec. Repetitive stimulation causes an electrical discharge of up to 1 mV. amplitude and 3 per sec. frequency in the habenular nucleus, which terminates with the panting syndrome after the end of stimulation but is not of the same frequency as the respiration. Direct heating of the habenular nucleus does not cause the panting syndrome until a damaging temperature is reached. Repetitive stimulation of a habenular nucleus for 5 min. can cause a 1°C. fall of rectal temperature at a room temperature of 18°C.

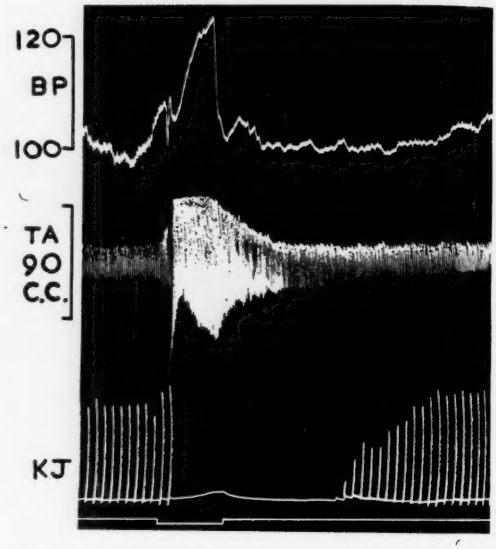

Fig. 1. Stimulation of the lateral habenular nucleus of a rabbit anæsthetised with urethane at 50/sec., 2·5 V., 4·2 m.sec. *BP*, blood pressure in carotid artery calibrated in mm. mercury ; *TA*, tidal air, expiration downwards ; *KJ*, knee jerk reflex, extension upwards, elicited at intervals of 5 sec. by a magnetic hammer

It is probable that the habenular, interpeduncular and dorsal tegmental nuclei and their tracts form an efferent pathway from the temperature sensitive region. This proposition is being tested by tracing degenerating fibres from small lesions in the temperature-sensitive region by the method of Nauta and Gygax.

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Composition of Some Fat-free Carcasses and Bodies of New Zealand Sheep

The composition of the fat-free carcass and body has been shown to be constant for mature animals of many species[1–5]. However, it is only recently that this constant has been confirmed for the sheep[5,6].

Data have accumulated in this laboratory on the composition of sheep carcasses covering the extremes in hot carcass weight and fatness likely to be met with in the New Zealand sheep industry (Table 1). The composition of the frozen carcasses was determined essentially by methods previously described[5], with the exception that ash determinations were carried out individually on the carcasses reported in this communication. The loss between hot and frozen carcass weight was assumed to be moisture when the composition of the hot carcass was being calculated. Data were also available on one newly born lamb and on three fœtuses which had been delivered by

Table 1. HOT CARCASS AND BODY COMPOSITION OF NEW ZEALAND EWES AND LAMBS

Carcasses*	Approximate age (months)	Hot carcass weight (lb.)		Fat per cent.		Per cent composition on fat-free basis					
						Water		Protein		Ash	
		Mean	Range	Mean	Range	Mean	S.D.	Mean	S.D.	Mean	S.D.
20 Southdown-Romney wether lambs	7	39·2	31·2– 43·8	31·6	24·7–40·0	74·6	0·7	19·9	0·7	5·6	0·5
40 ewes	27	63·4	27·0– 93·9	42·1	18·0–53·1	73·8	1·1	20·2	0·9	6·0	0·6
5 fat ewes	over 60	112·2	45·1–129·7	56·2	54·2–60·2	73·2	0·6	21·2	0·3	5·6	0·5
4 thin ewes	over 60	31·4	23·4–40·1	17·3	6·2–32·5	75·0	1·3	18·9	0·8	6·1	0·5
Fat ewe†	over 60	138·0	—	60·1	—	73·1	—	21·3	—	5·8	—
Thin ewe†	over 60	31·0	—	4·8	—	74·2	—	18·0	—	7·8	—
Oedematous ewe	over 60	30·3	—	2·0	—	82·2	—	13·0	—	4·8	—
Bodies*		'Body' weight (lb.)									
Ewe fœtus	Almost full term	5·7		3·8		80·5					
Ram fœtus	Almost full term	7·6		3·3		81·8					
Ewe fœtus	Almost full term	8·4		3·3		75·8					
Ewe lamb	New born	7·9		3·6		74·8					
Pregnant ewe	36	87·3		30·5		75·0		20·3		4·8	
Pregnant ewe	36	97·7		35·1		75·2		20·5		4·6	
Pregnant ewe	36	91·5		41·5		75·5		19·9		4·5	

* All carcasses and bodies are of the Romney breed unless otherwise stated.
† Selected as extreme carcasses from a commercial meat export works.

cæsarian section when they were within approximately one week of full term. These four bodies were analysed after the skin, tail, ears, and hooves were removed.

Also presented in Table 1 is the composition of three full-term pregnant ewe bodies which were analysed following the removal of the skin, hooves, fœtuses, non-fœtal uterine contents, and gastro-intestinal and urinary bladder contents. The slaughter procedure involved the loss of most of the blood.

It can be seen from Table 1 that in spite of a very wide range of carcass weights and degrees of fatness, the concept of constant composition of the fat-free carcass applied fairly well. Hopper[7] and Reid *et al.*[8] have shown in cattle that water percentage decreases and protein and ash percentages increase in the fat-free body with age. The present carcass and body data agree generally with this observation as far as protein and water percentages are concerned. No trend was noted with ash percentage, but the methods of ash determination used did not distinguish well between carcasses (unpublished data). However, an exception to the above hypothesis may be observed in the case of the thin ewes of which all but one contained much less fat than is normal in New Zealand Romney sheep of this age. These thin ewes had more water and less protein than the remaining aged animals. Increased hydration at lower levels of fatness has previously been noted for the sheep[9] and the pig[10].

Other factors thought to affect the composition of the fat-free carcass or body include thyroxine treatment[5], pregnancy[11,12], and œdema[13] resulting from undernutrition and disease.

The composition of the fat-free empty bodies of the pregnant ewes studied was similar to that derived by Californian workers[6] from data in the literature. The data analysed by them were apparently obtained from wethers and non-pregnant ewes of several breeds ranging in age from prematurely born lambs to mature sheep. Most of the animals contained less than 30 per cent body fat and a few emaciated sheep were also examined. It is suggested, therefore, that if the data analysed by these investigators are regarded as normal then there appears to be no effect of pregnancy on the body composition of the ewe.

In the present study the water content of the bodies was higher and the ash content lower than was the case for the carcasses. This difference can be explained by the fact that most of the bone (high ash, low water content) is in the carcass fraction of the body. The remaining bones which include those of the head, metacarpals, metatarsals, along with the bones of the feet, make up a small proportion of the non-carcass fraction of the body.

The financial assistance of the New Zealand Wool Board and Department of Scientific and Industrial Research is gratefully acknowledged. Data on the body composition of one lamb and three fœtuses were kindly made available by Dr. F. B. Shorland. Part of this work was carried out while one of us (M.J.U.) holds a New Zealand Wool Board Scholarship.

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Experiments on Wool from Copper-Deficient Sheep

WOOL from merino sheep suffering from severe deficiency of copper is very weak, has abnormal dyeing properties, and is difficult to process[1,2]. These characteristics are not easy to explain in terms of what is known about the structure and chemistry

of the fibres. Although deficiency of copper delays the rate of keratinization in the wool follicle[1], copper-deficient fibres appear to have the same structure as normal fibres and the differences between them revealed so far by chemical analysis (for example, differences in sulphur, cystine, cysteine, and N-terminal-residue concentrations[1,3,4]) do not offer a complete explanation for the large physical differences.

We have now found evidence from dissolution and fractionation experiments that copper deficiency in sheep is associated with a decrease in the proportion of certain sulphur-rich proteins in the wool fibres, which may possibly be responsible for some of the abnormal properties.

Experiments by Alexander and others on the dissolution and fractionation of wool keratin after mild oxidation have shown that the dissolved proteins may be divided into a major component with high molecular weight, low solubility in acid, and low sulphur content, and a minor component with low molecular weight, high solubility, and a high sulphur content; both being heterogeneous[5,6]. The following method was used to measure the proportions of these components in oxidized wool. A purified wool sample (0·100 gm.) was oxidized for 4·5 hr. at 5° in 2·5 c.c. of a performic acid mixture (9 vol. of 97 per cent formic acid to one of 30 per cent hydrogen peroxide[8]). After the acid had been sucked off, the sample was shaken at 30° with 20 c.c. of *p*H 6 pyridine-acetate buffer solution for 6–7 hr. The undissolved material, about 70 per cent of the original wool, was then filtered off, dried, and weighed; an approximate correction being applied for the increase in weight on oxidation. By this method it was possible to obtain reproducible values for the proportion of undissolved protein with a coefficient of variation of 7 per cent or less for a given wool. For wool from five copper-deficient merino sheep[4], the ratio of the weight of insoluble to soluble material ranged from 2·78 to 3·60; whereas for normal wool grown later by the same sheep when the copper deficiency had been corrected the ratios were from 2·32 to 2·83. The figures show that the copper-deficient wool contains, on the average, about 12 per cent less of the soluble component and correspondingly more of the insoluble component. Tests on wool from five merino sheep that had never experienced deficiency of copper gave ratios of from 2·24 to 2·42, which agrees with the above normal range. The values of the ratios depended slightly on the duration of oxidation, but the differences between different wool samples did not.

The soluble components isolated by the above procedure had, as expected, a higher concentration of cysteic acid residues than the whole fibre (for example, the soluble component from normal wool *E*5 contained 1300 μ mole of this residue per gm. compared with 800 μ mole per gm. in the rest of the fibre), although the difference was not as great as that found after other methods of separation[7].

The source of the 'soluble' and 'insoluble' components in the original fibre is not known. They do not, for example, appear to be directly correlated with bilateral structure. Nevertheless, the above results may be understood in terms of the often-postulated theory that keratin contains a low-molecular-weight sulphur-rich "intermolecular cementing substance[5,9]", with the additional assumption that this plays a large part in determining the physical properties of the fibres. According to this, copper-deficient wool is weaker and is more easily penetrated by reagents than normal wool because it has less of this substance. There is yet no direct evidence for these assumptions; but it is conceivable that during the abnormally long keratinization of copper-deficient wool some low-molecular-weight material is lost to the fibre because it is not involved in disulphide bonding at a fast enough rate.

A full account of these experiments will, it is hoped, be reported later.

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Influence of the Thymus upon the Egg-White Allergy in the Rat

It has been shown previously that the thymus is extensively involved in the interaction between the anterior pituitary and the adrenal cortex[1]. Thus, the thymus was found a moderator of the corticotropic influence[2], whereas it enhanced the influence of growth hormone[3]. The thymus was shown to be a synergist to deoxycorticosterone[4]. Furthermore, thymus atrophy following hypophysectomy was prevented with growth hormone injections[5], and the changes of the thymus resulting from adrenalectomy was prevented by deoxycosterone (not by cortisone). These involvements of the thymus in the interaction between anterior pituitary and adrenal cortex made it probable that the thymus may exert an influence upon the course of inflammatory reaction.

In experiment 1, 15 female albino rats weighing 120–140 gm. were used. They came from a local inbred strain, bred for 5 generations in our laboratory. The animals were treated as follows: 5 were kept as normal controls; 5 were thymectomized with the technique of Segaloff[6] and injected daily with 1 c.c. of Ringer's saline for 10 days from the day after the operation.

5 were thymectomized and injected daily under the same conditions with the thymus extract of Bezssonoff and Comsa[1] (1958) at a dose of 200 units[7].

On the 11th day after the thymectomy, all 15 animals were injected intraperitoneally with 2 c.c.[3] of crude hen egg-white, as indicated by Selye[8].

Experiment 2 was performed under the same conditions in hypophysectomized rats (hypophysectomy 48 hr. after the thymectomy).

Experiment 3 was performed under identical conditions upon 3 groups of adrenalectomized rats (the adrenalectomy was performed in the usual way, (see ref. 7) 48 hr. after the thymectomy).

As described by Selye, the normal rats developed a severe shock about 1 hr. after the injection of egg-white. This shocked condition aggravated gradually. It was fully developed about 1 hr. later. The muzzle, the ears and the paws were swollen and intensely red; they became gradually cyanotic. During the second hour, the animals were drowsy, flaccid and algid. They breathed heavily and the pulse-rate was decreased. Normal rats of our strain recovered from this shock during the following 6–10 hr.

In thymectomized animals, the reaction was reduced to a slight congestion of the paws. The muzzle was not visibly affected. Neither dyspnœa, nor algidity was noted, and the behaviour of the animals was strictly normal. Thymectomized animals injected with thymus extract developed a peculiarly intense reaction. Two of those died respectively 110 and 140 min. after the egg-white injection.

The observations made in experiment 2 (hypophysectomized rats) were similar to those of experiment 1. In this group, too, the egg-white shock was peculiarly mild in the thymectomized group. The severe reaction could be restored in those animals by previous injections of thymus extract, as in experiment 1.

In adrenalectomized rats (experiment 3), the egg-white shock was peculiarly severe in all animals, whether thymectomized or not or thymus-injected. All those animals died during the 4 hours following the egg-white injection.

Thus the reaction to egg-white is noticeably decreased following thymectomy. The normal reaction is at least restored by means of injection of the thymus extract of Bezssonoff and Comsa[1]. It can be concluded that the thymus exerts a prophlogistic influence in the rat and that this influence is supported by a substance present in the extract of Bezssonoff and Comsa.

Previously it was found that the healing of the thymectomy wound is difficult in guinea pigs. A torpid suppuration (recalling caseification) is frequent (which is never seen following other operations in guinea pigs). This suggested that the resistance against infection might be disturbed by thymectomy. This may be understood as a consequence of the decreased prophlogistic influence in thymectomized animals.

This influence of the thymus cannot be understood entirely as an expression of the synergistic effect of the thymus to the growth hormone of the anterior pituitary[2], since it could be observed in hypophysectomized rats as well. Partly at least it may be understood as an expressing the interference of the thymus with an antiphlogistic influence still present after hypophysectomy, but suppressed by adrenalectomy. It is difficult to imagine the mechanism of this influence, as it is generally admitted that exclusion of the corticotropic pituitary factor (by means of hypophysectomy) results in a suppression of the cortisone as well.

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Visual Pigments of Some Common Laboratory Mammals

Recently[1], homogeneous visual pigments ranging in λ_{max} from 491 mμ to 501 mμ have been obtained from a number of different mammalian retinæ. The present work deals with the homogeneity and spectroscopic properties of visual pigments found in some common laboratory mammals.

Alkaline (*p*H 8·3–8·8) aqueous digitonin extracts were prepared either from the acid-washed dark-adapted whole retinæ[2] or from the alum-hardened rods[3]. The contained visual pigments were then tested for homogeneity by the method of partial bleaching[2].

Each species was found to possess one visual pigment, the λ_{max} of which was determined by two methods. The first method consisted of directly determining the λ_{max} of the absorption spectrum and correcting this for the effect of extraneous absorption by impurities[3]. Solutions requiring a correction of 1–2 mμ only were used for this purpose. The second method involved measuring the separation of the alkaline difference spectrum, plotted on a frequency scale[4], from that of a known visual pigment, that is, frog rhodopsin, $\lambda_{max} = 502{\cdot}6$ mμ[3].

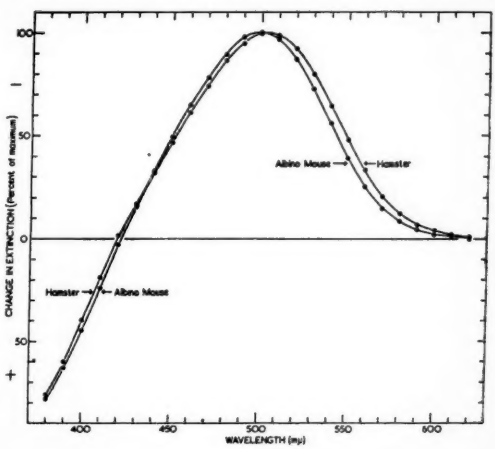

Fig. 1. Difference spectra for albino mouse and hamster visual pigments (at *p*H 8·75 and 8·64 respectively)

Table 1

Species	λ_{max} of Visual pigment (mμ)	
	Absorption spectrum data*	Difference spectrum data†
Albino rat	498±1 (1)	498·4±0·5
Albino mouse	—	498·0±0·5
Albino guinea pig } Pigmented guinea pig }	497±1 (1)	497·5±0·5
Pigmented rabbit	502±1 (2)	502·1±0·5
Hamster	502±1 (1)	502·3±0·5
Rhesus monkey	—	497·0±1

* Figures in parenthesis indicate the magnitude (in mμ) of the correction used.

† Maxima calculated on the basis of frog rhodopsin $\lambda_{max} = 502{\cdot}6$ mμ.

The summarized results are shown in Table 1 (see also Fig. 1). All measurements, with the exception of rhesus visual pigment, are at 25±0·5°C. The λ_{max} of the rhesus visual pigment was obtained from measurements made at 37°C. (the bleaching of rhesus visual pigment at 20°C. is complicated by the

presence of a semi-stable photoproduct, $\lambda_{max} \simeq 480$ mμ. No trace of this substance can be detected at 37°C.).

Photosensitivity measurements (carried out according to the Dartnall's method[5]) show that rat and hamster visual pigments are about 10 per cent more photosensitive at their respective maxima than is frog rhodopsin at 500 mμ.

Previous workers[3], who did not test for homogeneity, found that the albino rat visual pigment had $\lambda_{max} = 498{\cdot}2 \pm 0{\cdot}4$ mμ, in good agreement with the present work.

It is interesting to note that single visual pigments are obtained both from the rod-pure retina of guinea pig and the mixed retina of rhesus, and that these pigments are spectroscopically extremely similar (λ_{max} within 497 ± 1 mμ). Similarly, frog (mixed retina) and rabbit (nearly rod-pure retina) respectively yield single pigments differing by no more than 0·5 mμ in λ_{max}.

In two mammals, rhesus and hamster, retinal removal and extraction were sufficiently quantitative to enable an estimate to be made of the nominal *in situ* density of visual pigment[6]. In rhesus this was 0·03 at 497 mμ (cf. the figure of 0·016 obtained with human[6]); in hamster at 502 mμ the *in situ* density was found to be 4–6 times greater, 0·14–0·19.

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PLANT PHYSIOLGOY

Electrical Determination of Transport of 3-Indole Acetic Acid in *Avena*

Recent experiments in this laboratory have shown that an electrical potential wave travels down a decapitated *Avena sativa* coleoptile as a result of applying 3-indole acetic acid to the top. A similar wave is observed when the top of an intact coleoptile is illuminated.

A technique was developed in order to provide reliable measurements of electric potentials on *Avena*. A modification of the liquid drop method of contact with the coleoptile was used. The probe consisted of two concentric glass tubes with an outer tip diameter of 0·4 mm. The contact solution (10^{-2} molar potassium chloride) was supplied down one tube and was sucked away through the other at such a rate that the solution in the 0·2-mm. wide 'bridge' between probe and plant was changed several times per second. The solution then flowed past the mercury-calomel electrode connected to the electrometer. This flowing solution ensured constant conditions at the point of contact with the plant and enabled highly reproducible results to be obtained. The probe tip could be placed at any level on one side of the coleoptile and measurements made of the potential between that probe and a reference probe (also with flowing solution) at the base of the coleoptile. All measurements were made in red light, in a saturated atmosphere at 25°C.

The potential difference between any pair of points on an intact coleoptile was found to undergo oscillations of a few millivolts magnitude and about 15 min. period. When the coleoptile was decapitated in the manner of the *Avena* test for plant hormones[1] these oscillations were largely suppressed for some hours. Half an hour after the second decapitation (the primary leaf having previously been removed), an agar block containing 2 mgm./l. of 3-indole acetic acid was applied to the cut top of one of these auxin-deficient and electrically 'quiet' coleoptiles. A series of well-defined variations was afterwards observed in the electric potential gradients down the coleoptile.

Fig. 1 shows the time variations, averaged for six plants, of the potentials across successive 2-mm. lengths of tissue 2–12 mm. below the cut top of the coleoptile. $V_2 - V_4$ is the mean potential difference between the points at 2 mm. and 4 mm. below the cut top. The time zero is the instant of application of the 3-indole acetic acid. The characteristic feature of each potential graph is a peak followed by a trough which usually precedes a damped oscillation. The fall from the peak to the trough in the potential gradient is, in all 5 cases, statistically significant at the 1 per cent level. The spacing down the figure of the five time axes represents 2 mm. intervals down the coleoptile, and it is seen that the times on these axes of the peaks form a straight line in Fig. 1. The position of the peak falls 8 mm. in 34 min., indicating a rate of transport for the electrical phenomenon (or its cause) of 14 mm. per hour. Extrapolation of the line shows that its intercept on the time axis at the top of the coleoptile is 3 min. after the application of the 3-indole acetic acid. Three coleoptiles on which agar blocks containing no 3-indole acetic acid were placed showed, over the hour of observation, no sign of the highly significant variations in the potential gradients of the figure. Went and White[2] report a rate of transport

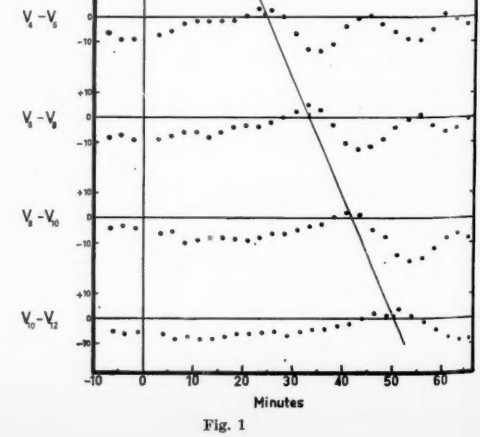

Fig. 1

of 3-indole acetic acid of 12·4 mm. per hour. It is suggested that our observed variations in the potential gradients down the coleoptile are an effect of the distinct advancing front of the stream of 3-indole acetic acid from the agar block moving at a constant rate of 14 mm. per hour.

The experiments described above were suggested by the observation of an electrical wave which travelled at a similar rate down intact dark-grown coleoptiles upon illumination with white light. The illumination was limited to the apical 2 mm., with an intensity of 50 metre-candles. It appeared that in this case a light-induced discontinuity in the auxin stream from the coleoptile apex was being observed by its electrical effect on the tissue it reached.

The results will be published in detail elsewhere.

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RADIOBIOLOGY

Radioprotective Action of Methoxamine

Several vasoconstrictor compounds have been shown to protect animals against lethal doses of X-radiation. A review of this field has recently been prepared by Pihl and Eldjarn[1]. Methoxamine, β-hydroxy, β-(2,5-dimethoxyphenyl)-*iso*-propylamine, is a compound with pronounced vasoconstrictor properties[2] which does not appear to have been previously tested against radiation. We wish to report the radioprotective action of methoxamine hydrochloride ('Vasoxine', Burroughs Wellcome) in mice.

Female *R* mice (a cross between *PCT* female and *C3H* × 101 male) were used in the experiments. They were irradiated from below in groups of five in their living cage as follows: 250kV., 14 m.amp.; h.v.l. 1·2 mm. copper distance to under side of cage 60 cm.; dose-rate 71·6 rads per minute. Control and treated mice were chosen at random and irradiated in the same box. The LD_{50} of methoxamine hydrochloride in female *R* mice was found to be approximately 75 mgm./kgm. intraperitoneally.

The results of a preliminary investigation, in which all the mice were irradiated with the estimated LD_{97} of X-rays for this strain, can be seen in Table 1. The best protection was obtained with 25 mgm./kgm. intraperitoneal of methoxamine hydrochloric acid if the compound was injected 5 or 15 min. before irradiation. The protective effect was almost halved when the mice were injected 60 min. before irradiation. Smaller doses of methoxamine produced correspondingly less protection.

Table 2 presents the results of experiments from which the dose reduction factor can be deduced. The LD_{50} of radiation for *R* mice is 825 rads for a 30-day period post irradiation. 25 mgm./kgm. intraperitoneal of methoxamine hydrochloride given 15 min. before irradiation raised the LD_{50} to 1100 rads, that is, a dose reduction factor of 1·33. Even if the dose of methoxamine is increased to 50 mgm./kgm. there is no increase in the degree of radioprotection.

Also reported in Table 2 are the results of two experiments in which mice were pretreated with compounds that are chemically related to methoxamine. Ephedrine, β-hydroxy, β-phenyl, N-methyl-*iso*-propylamine, did not protect mice against 1000 rads in the dose used. This finding agrees with those of Bacq[3] and Langendorff and Koch[4], with different doses of ephedrine. Propadrine, β-hydroxy, β-phenyl-*iso*-propylamine, was also tested against 1000 rads and found to have no protective effect. Chemically, propadrine differs from methoxamine only in the absence of methoxy groups in the 2 and 5 positions of the benzene ring.

Table 1. Protective Effect of Methoxamine Hydrochloride given to Mice before 950 rads

Treatment	Time of injection (min.)	No. living / No. in experiment	Percentage 30 days survival
Methoxamine, 1 mgm./kgm. i.p.	15	0/10	0
Methoxamine, 10 mgm./kgm. i.p.	15	7/10	7
" "	60	1/10	10
Methoxamine, 25 mgm./kgm. i.p.	5	9/10	90
" "	15	8/10	80
" "	60	5/10	50
Saline controls	15	1/35	3

Table 2. Protective Effect of Methoxamine Hydrochloride in Mice Irradiated with High Doses of Radiation

Treatment	X-rays (rads)	Time of injection (min.)	No. living / No. in experiment	Percentage 30 days survival
Methoxamine 25 mgm./kgm. i.p.	1000	5	8/10	80
" "	1100	5	8/20	40
" "	1200	5	3/20	15
Methoxamine 25 mgm./kgm. i.p.	1000	15	16/20	80
" "	1100	15	10/20	50
" "	1200	15	3/20	15
Methoxamine 50 mgm./kgm. i.p.	1100	15	9/20	45
" "	1200	15	3/20	15
" "	1300	15	0/20	0
Saline Controls	1000	15	0/20	0
" "	1100	15	0/20	0
" "	1200	15	0/20	0
Ephedrine HCl 100 mgm./kgm. i.p.	1000	15	0/10	0
Propadrine HCl 50 mgm./kgm. i.p.	1000	15	0/10	0

Hope has shown that many radioprotective substances have the common action of lowering the body temperature of mice[5,6,7]. Using his apparatus we measured the rectal temperature of a group of five mice after 25 mgm./kgm. intraperitoneal of methoxamine hydrochloride. The average rectal temperature fell steadily to a minimum of 32°C. 45 min. after injection.

The mechanism of the radioprotective action of methoxamine is not known. However a possible explanation is that it acts by producing vasoconstriction and anoxia in radiosensitive tissues, as has been suggested by van der Meer and van Bekkum to be the case with several biological amines[8]. A pharmacological explanation, rather than one in which the amine competes with tissue constituents for radiation induced free radicals[9], seems to be indicated by the fact that methoxamine is no more active at 50 mgm./kgm. than it is at 25 mgm./kgm. The pharmacological action of methoxamine is primarily one of vasoconstriction, resulting in increased peripheral resistance; whereas that of ephedrine and propadrine is principally a direct effect on the heart[10].

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HÆMATOLOGY

Fœtal Hæmoglobin in the Monkey

A number of well-defined physico-chemical differences exist between human adult and fœtal hæmoglobins. These include their electrophoretic and chromatographic behaviour under defined conditions, a large difference in alkali denaturation rates, and the changed position and contour of the tryptophan fine-structure band.

The differences between the fœtal and adult hæmoglobins of such other mammals as have been examined, also show differences in electrophoretic and chromatographic mobility and alkali denaturation rates—in the latter case generally in the opposite sense to the human pigments[1]. Jope[2] showed that the shift in tryptophan band wave-length did not extend to any of the animal fœtal pigments which he examined. We have been concerned to establish whether the unique specific differences between human hæmoglobins *A* and *F* occur in other primates, especially since one important attribute of human adult hæmoglobin (the presence of a minor component hæmoglobin A_2) is found in some, though not all, species of monkey[3].

Electrophoretic behaviour in agar gel has been examined. At *p*H 6·2[4] human hæmoglobin *F* migrates much more rapidly than hæmoglobin *A* and gives a distinctive straight front. Fig. 1 shows that the hæmoglobins of the adult and fœtal rhesus monkey (*Macacus rhesus*) migrate in the reverse order, but that the characteristic zone shape is retained. Under these conditions the human and monkey fœtal pigments can also be separated without difficulty.

The position of the tryptophan fine-structure bands in the pure fœtal proteins has been established by direct examination of the zones in the gel with the aid of the moving-plate spectrograph[5]. Fœtal rhesus monkey hæmoglobin has its band at about 289·7 mμ compared with 289·6 mμ for the human fœtal and 291·0 mμ for both adult pigments. There is thus a close similarity between the monkey and human pigments in this unique respect. Alkali denaturation has also been studied, and it is found that monkey fœtal hæmoglobin has a greater alkali resistance than adult. When followed photometrically with a red filter (Wratten 29) the difference is of a similar order to that found in the corresponding human proteins, but the rate curve for adult monkey hæmoglobin deviates from linearity.

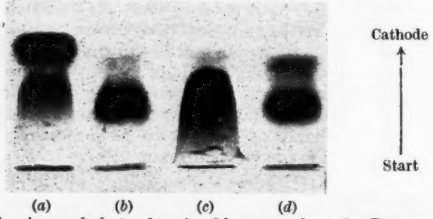

Fig. 1. Agar gel electrophoresis of human and monkey hæmoglobins at *p*H 6·4.
a, human hæmoglobins *A* + *F*.
b, *d*, monkey cord blood hæmoglobins.
c, maternal monkey hæmoglobin.

Further investigations on the fœtal pigments are in progress, and it is hoped to examine those of other primates if they can be obtained.

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Synthesis of Hæm by Circulating Blood Cells

It has been shown that hæm containing iron-59 can be crystallized from the reticulocytes of blood samples to which iron-59 has been added prior to incubation at 37°C.; but the mature mammalian erythrocyte does not synthesize hæm in this way[1,2]. The results of an investigation of the nature and kinetics of this phenomenon in reticulocytes and avian cells suggests that there are at least two phases; first the entry of iron into the cell and secondly the synthesis of hæm from this iron.

Blood was collected from rats five days after intraperitoneal injection of 2 per cent phenylhydrazine solution, and from other rats three days after removal of 5 ml. of blood by cardiac puncture. Human reticulocytes were obtained from patients with hæmolytic anæmia or with pernicious anæmia after treatment, while cardiac puncture of pigeons provided avian nucleated cells. Aliquots of 1 ml. of blood were placed in small vaccine vials sealed with rubber bungs and the vials were rotated three times per minute during incubation at 37°C. The solution of ferric chloride labelled with iron-59 solution was of high specific activity and was added in 0·1-ml. quantities containing approximately 0·5 μc. Before assay of radioactivity, the red cells were washed ten times with physiological saline solution and hæmolysed by the addition of distilled water. Hæm was extracted from the hæmolysates by Heilmeyer's method[3] and the radioactivity of the hæm and non-hæm fractions was measured.

The principal findings were as follows:

(1) In mammalian blood, approximately 90 per cent of the total uptake occurred within 2 hr. and hæm synthesis took place rapidly. The ratio of hæm iron-59 to non-hæm iron-59 in all hæmolysates was approximately 65 to 35. Uptake and hæm synthesis were also parallel in avian blood, but the rate was slower and was related linearly to time up to 8 hr.

(2) Incubation of blood prior to the addition of iron-59 reduced the total uptake of iron-59 and also its percentage in the hæm fraction. A typical

protocol is shown in Table 1. The addition of glucose, inosine or inosine triphosphate did not prevent the effect of preincubation. On the other hand, blood kept for 22 hr. at 4°C. showed no impairment of uptake of iron-59 when returned to 37°C.

Table 1. EFFECT OF PRELIMINARY INCUBATION ON THE UPTAKE OF IRON-59 AND SYNTHESIS OF HÆM BY MAMMALIAN RETICULOCYTES

Incubation before addition of iron-59 (hr.)	Iron-59 in sample (mean c.p.m. of 3 samples)	Iron-59 in hæm fraction (mean c.p.m. of 3 samples)	Iron-59 in hæm fraction (per cent of uptake)
0	48,000	29,760	62
2	36,400	27,472	48
4	40,700	22,792	44
8	10,600	1,908	18

(3) Iron-59 entering the reticulocytes was not removed by washing the cells ten times with physiological saline solution or with 2 per cent solution of the disodium salt of ethylenediaminotetracetic acid.

(4) Uptake was impaired by the presence of 10^{-3} *M* mercuric chloride and by preliminary exposure (followed by saline washing of the cells) to solutions of iron salts. It was not changed by 10^{-3} *M* iodoacetate or 10^{-3} *M* potassium cyanide solutions.

(5) By contrast, synthesis of hæm from iron which had entered the cell was markedly reduced by 10^{-3} *M* potassium cyanide but was not affected by the other agents.

(6) Oxidative respiration of blood samples was reduced to 71 per cent of the control with 10^{-3} *M* iodoacetate, with the sample containing $1{\cdot}92\times10^{9}$ reticulocytes; to 55 per cent by 10^{-3} *M* mercuric chloride with $2{\cdot}16\times10^{9}$ reticulocytes; and to 16 per cent by 10^{-3} *M* potassium cyanide with $1{\cdot}30\times10^{9}$ reticulocytes.

The results are consistent with the hypothesis that entry of iron into the reticulocyte is dependent on some property of the cell, possibly of the surface. The same property is not present in the mature erythrocyte and is reduced by incubation of reticulocytes at 37°C. It is likely that iron entering a cell is rapidly incorporated into protoporphyrin which has already been synthesized. The amount of free protoporphyrin in the reticulocyte is greater than in the mature cell[4], and the reduction suggests that hæm is synthesized from the preformed protein moiety. This hæm synthesis is a cyanide-sensitive process and is reduced by preliminary incubation of the cell, but its nature has not yet been elucidated. In contradistinction to the rapid uptake in the mammalian reticulocyte, the steady and continuing rate of entry of iron into the avian cell raises the possibility that protoporphyrin synthesis is a continuous process in these cells. As prior incubation of reticulocytes with non-isotopic iron reduces the uptake of iron-59, the amount of free protoporphyrin may be a factor determining the amount of iron-59 which enter an otherwise susceptible cell.

The role of non-hæm iron[5,6] has not been investigated in these experiments, and it is uncertain whether it is a stage in the synthesis of hæm or whether iron entering the cell can combine directly with protoporphyrin to form hæm and with some other substance to form the non-hæm fraction. It has been shown that when cells are incubated before the addition of iron-59, or when potassium cyanide is added with the iron-59, synthesis of hæm is impaired and the proportionate amount of iron in the non-hæm fraction rises. The non-hæm fraction is therefore unlikely to be a limiting factor in the synthesis of hæm.

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PATHOLOGY

Effect of Steroids and Related Compounds on the Growth of Dermatophytes

IT has been suggested that steroid hormones may influence the course of a dermatophyte infection indirectly either by decreased resistance as during cortisone therapy[1] or at puberty in *Microsporum* infections[2,3]; a more direct fungistatic action is suggested by reports of *in vitro* inhibition by steroids or related compounds[4–8]. That steroids may have a direct effect upon growth and metabolism of fungi was particularly suggested by work with *Neurospora crassa*[9]. Since the skin is a site of appreciable steroid synthesis and its metabolism is known to be affected by a number of steroid hormones it seemed that unsolved problems of susceptibility to dermatophyte infection, development of chronic infections by some individuals and the localization of infection in the epidermis or hair root may be explicable in terms of direct or indirect action of steroids upon the fungi.

As a preliminary study the effect of a number of steroids upon *in vitro* growth of representative strains of dermatophytes has been made. Effect on growth was assessed in terms of mycelial dry weight produced during growth in 100 ml. conical flasks over 7 days at 33° in 10 ml. liquid synthetic medium in stationary culture. Steroids were added in chloroform-ethanol (1:1) solution to sterile flasks and after evaporation of the solvent sterile medium was added. The inoculum was 0·5 ml. of homogenized washed mycelium suspended in sterile water and grown initially in Sabouraud maltose broth. Initially a survey was made of the effect of a number of steroid hormones and of steroids and related compounds formed in skin tissues upon a strain of *Trichophyton rubrum* (Table 1).

Table 1. EFFECT OF STEROIDS ON GROWTH OF *T. rubrum*, STRAIN *OS*

Compound	Mean % inhibition or stimulation	Compound	Mean % inhibition or stimulation
DL-mevalonic acid	−7	Deoxycorticosterone	91
Squalene (90 per cent)	63	Deoxycorticosterone acetate	47
Lanosterol	−5	Compound S	−4
Cholesterol	−18	Cortisone	−3
Progesterone	70	Cortisone acetate	12
11α-hydroxy progesterone	59	Hydrocortisone alcohol	−1
epi-œstradiol	39	Hydrocortisone acetate	4
β-œstradiol	39	Calciferol	79
Stilbœstrol	72	7-dehydrocholesterol	77
Testosterone	77	7-dehydrocholesterol acetate	16

Growth medium: 21 mgm. L-glutamic acid, 18 mgm. potassium dihydrogen phosphate, 1 mgm. crystalline magnesium sulphate, 100 mgm. D-glucose, 20 μgm. biotin, 200 μgm. thiamine hydrochloride, 200 μgm. *i*-inositol; dissolved in water and with the inoculum, vol. made to 10 ml. Growth for 7 days at 33° as described in text. Results expressed as per cent inhibition (+ve) or per cent stimulation (−ve) of growth in the presence of 250 μgm. 'steroid'/ml. medium compared with growth obtained in the absence of steroid. Mean values given are those for 4 experiments, each experiment being carried out in triplicate.

A selection was then made from these compounds of a group of materials representing those inhibiting growth and those which produced no significant effect and these were tested against a further strain of *T. rubrum* and representative strains of other dermatophytes (Table 2).

Table 2. Inhibition of Growth of Representative Strains of Dermatophytes

Compound	*T. rubrum* strain *M*	*Microsporum canis* J.Mc.A.	*Trichophyton mentagrophytes*	*Epidermophyton floccosum*
Cholesterol	−7	−8	−5	22
Squalene	46	24	19	52
Deoxycorticosterone	95	92	97	77
Deoxycorticosterone acetate	57	−3	44	11
Cortisone	9	−2	16	7

Growth conditions and evaluation of results as in Table 1.

It thus appears that there is a group of steroids which strongly inhibit the growth of dermatophytes and the most active steroid tested in this group is desoxycorticosterone, a similar finding to that of Lester *et al.*[9] for *N. crassa*. The inhibitory activity does not appear to be related to physiological function of the steroids in mammals. There is a second group, exemplified by cholesterol and cortisone, which exert no inhibiting action when tested at the same level. It may also be noted that the less polar acetates show a marked reduction in inhibiting power. It thus appears that steroids may have a considerable effect upon fungal growth and further studies into the nature of their action is in hand.

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VIROLOGY

Recovery of Infectious Nucleic Acid from Tobacco Mosaic Virus inactivated by Antibody

Although the combination of antibody with tobacco mosaic virus is reversible[1], it does not necessarily follow that dissociating the antibody restores infectivity. Inactivation could be permanent despite the reversibility of the antibody - virus reaction. Several reports have indicated, however, that antibody inactivation of tobacco mosaic virus is not entirely irreversible.

Some recovery of infectivity was reported by Bawden[2] following dilution of a mixture of antiserum and tobacco mosaic virus. Enzyme digests of antibody - tobacco mosaic virus complexes have also been used with partial success in attempts to recover infectivity[3]. Nevertheless, the amounts of the virus reactivated in these experiments were small and variable, leaving open the possibility that much of the virus may have remained irreversibly killed.

In the present work, a competitive, non-infectious, antigen was added to the antiserum - virus mixtures as an alternative to the methods of dilution and digestion for the removal of antibody. Since the antibody - virus reaction is reversible, competition should affect a new equilibrium in which the antibody is re-distributed in such a way that a fraction of the virus may be reactivated.

The results of a competitive antigen experiment using tobacco mosaic virus inactivated by ultra-violet light (which retains the same capacity to bind antibody as unirradiated virus but does not compete with viable virus in causing infection) is shown in Table 1. The results do not suggest that any infectivity was recovered by this technique, although the amount of inactivated virus added after the first incubation period was as much as 40 times the initial concentration of the live virus. Using tobacco mosaic virus protein sub-units as the competing antigen also failed to restore activity.

Table 1

Reaction 1*		Reaction 2	
TMV conc.	Serum conc.	Ultra-violet TMV conc.	Virus infectivity surviving
(mgm./ml.)	(ml./ml.)	(mgm./ml.)	(per cent)
2×10^{-3}	4×10^{-4} (N.S.)	4×10^{-3}	100
2×10^{-3}	4×10^{-4}	PO_4	6·5
2×10^{-3}	4×10^{-4}	4×10^{-3}	6·2
2×10^{-3}	4×10^{-4}	2×10^{-3}	4·8
2×10^{-3}	4×10^{-4}	1×10^{-3}	7·4

* Antiserum or normal serum (N.S.) was incubated with tobacco mosaic virus for 1 hr. at room temperature. Following this incubation an equal volume of tobacco mosaic virus irradiated with ultra-violet light (inactivated to 10^{-3} survival) was added. This second reaction was allowed to proceed for 2 hr. before the residual infectivity was assayed.

In further competition experiments, a fraction of the activity was restored by acidifying the reaction mixtures to *p*H 3·3–3·5. At this low *p*H, the dissociation contant for the antibody-antigen reaction is increased, and more than half the combined antibody is released. Furthermore, acidification was shown to have no effect on the infectivity of the virus controls, nor did it impair the combining power of the antibody. Assaying the infectivity following neutralization, suggested that a measurable amount of the previously inactivated virus could be reactivated (see Table 2).

Table 2

Reaction 1*		Reaction 2		
TMV conc.	Serum conc.	Ultra-violet TMV conc.		Virus infectivity surviving
(mgm./ml.)	(ml./ml.)	(mgm./ml.)		(per cent)
2×10^{-3}	10^{-3}	4×10^{-3}	acidify and neutralize	7·9
2×10^{-3}	10^{-3}	4×10^{-3}	control	2·5
2×10^{-3}	10^{-3} (N.S.)	4×10^{-3}	acidify and neutralize	100
2×10^{-3}	10^{-3} (N.S.)	4×10^{-3}	control	100

* Antiserum or normal serum (N.S.) was incubated with tobacco mosaic virus for 1 hr. after which tobacco mosaic virus inactivated by ultra-violet light (inactivated to 10^{-3} survival) was added. In reaction 2, aliquots from the first reaction were acidified to *p*H 3·5 and held at this acid *p*H for 2 hr. Following this they were neutralized and allowed to stand for one more hour at room temperature. The control samples remained neutral and at room temperature for the same length of time. All samples were assayed for residual infectivity at the same time.

These experiments have been repeated many times. Reactivation after acidification and neutralization has been observed in some experiments, but not in others performed in an identical manner. It appears then, from these experiments and those of others, that once antibody has been in combination with virus, recovery of activity is a difficult proposition at best. Whether this failure to recover any substantial infectivity is the result of improper or faulty technique, or of a permanent alteration in the virus structure is at present unknown. Nevertheless, the opportunity exists for determining whether any

alteration, if present, resulting from antibody attachment, is restricted to the protein coat, or extends to the nucleic acid within the rod.

In order to ascertain whether the infectivity of the nucleic acid was impaired or not after antibody had inactivated the virus, the nucleic acid was extracted using cold phenol according to Gierer and Schramm's method[4]. The infectivity of this extracted nucleic acid was compared with identical extracts from virus treated with normal serum, and virus in neutral *M*/15 phosphate buffer. Each treatment was assayed for residual virus infectivity before the nucleic acid preparation was made. The results are presented in Table 3.

Table 3. TOTAL NUMBER OF LESIONS PRODUCED ON 12 LEAVES OF *Nicotiana glutinosa*

Treatment*	Nucleic acid extract	Unextracted virus
Antiserum 17–18	521	61
Normal serum	686	1042
Phosphate buffer	596	990

* Serum diluted 1/5 was added to an equal volume of tobacco mosaic virus strain *U2* at 1 mgm. protein/ml. Aliquots for residual virus infectivity were diluted 1/1000 before assay. The nucleic acid extracts were assayed without dilution.

The results show that whereas the antiserum had inactivated the virus to less than 10 per cent survival, no such marked reduction in the infectivity of the nucleic acid extracted from the inactivated virus was evident. The results of two additional experiments confirmed the above finding. Thus, the effect of antibody on the virus is restricted to some function associated with the protein coat, since the nucleic acid extracted from virus inactivated by antibody is as infectious as nucleic acid extracted from virus treated with normal serum or phosphate buffer. These observations are in accord with those of Ada and Anderson[5] who extracted infectious nucleic acid from Murray Valley encephalitis virus after destroying much of the infectivity of the virus by a variety of different physico-chemical treatments.

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HISTOLOGY

Histological Localization of Fluorescent Thyroid-stimulating Hormone in Rat Tissues

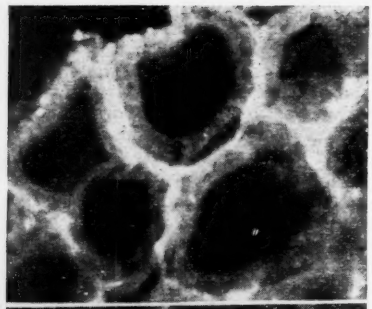
SONENBERG *et al.*[1] have studied the fate of preparations of thyroid stimulating hormone injected into animals by the use of hormone preparations labelled with sulphur-35, and subsequent determination of the distribution of the radioactive sulphur. After its introduction into the circulation of the experimental animal, thyroid stimulating hormone was distributed widely, significant concentrations of radioactivity being observed in the thyroids, gonads, liver, retrorbital tissues and possibly muscle[2,3].

In an attempt to determine the localization histologically, Armour thyroid stimulating hormone (lot *U* 3502) was labelled with lissamine-rhodamine *B* 200 by a modification of Chadwick's method for serum proteins[4]. As a result of this labelling 0·1537 μmole of fluorescent dye was bound to 1 mgm. of the hormone. The biological activity of the labelled hormone was checked in newly born chickens following the method of Gilliland and Strudwick[5] and it was found that after labelling thyroid stimulating hormone lost 56 per cent of its activity.

20 mgm. of labelled thyroid stimulating hormone in 0·3 c.c. saline solution was injected intravenously into rats weighing 100 gm. After the extraction of blood samples by aortal puncture, the animals were killed 5, 10, 20, 30, 45 and 60 min. after the injection. The electrophoretic patterns of these blood samples showed the labelled hormone to be present in the α- and β-globulin fractions.

All tissues were fixed in 10 per cent formaldehyde and embedded in paraffin following the usual techniques. Sections were cut at 10 μ and mounted with 'Permount'. The different tissues were examined with a Leitz 'Dialux' fluorescence microscope using a *UG* 2 mm. filter which provides a wave-length of approximately 400 mμ. The distribution of the fluorescence was as follows: 5 min. after injection it was present in the lumen of arteries and capillaries, in the internal and elastic membranes and in the tunica adventitia of arterioles. It decreased from these sites after 10 min. and disappeared 30 min. after the injection. In the thyroid gland fluorescence was present after 5 min. in the lumen of capillaries, in the basal membranes and in the basal region of the follicular cells. It disappeared completely after 10 min. In the ocular tissues, beginning after 10 min. and increasing up to 30 min., fluorescence was observed in the connective tissue of eyelids, sclerotica, ciliary processes, interfibrilar connective tissue of eye muscles and in the lacrimal gland cells. Fluorescence was also observed in the interfibrilar connective tissue of skeletal muscles, perivisceral adipose tissue, mast cells of adipose and loose connective tissues, and macrophages of liver, spleen and lymphatic nodes. After 30 min., fluorescence disappeared from almost all tissues but still persisted after 60 min. in Kupffer cells and macrophages of bone marrow, spleen and lymphoid nodes. In the gonads, the Leydig cells of testes and granulosa cells of the growing ovarian follicles showed fluorescence 10–60 min. after the injection. In the kidney after 5 min. fluorescence was present in the lumen of glomerular capillaries, in the lumen and brush border of proximal convoluted tubules and with greater intensity in the lumen and in the cells of distal convoluted tubules, as well as in the papillary ducts. Between 5 and 60 min. fluorescence increased progressively in the cytoplasm of the proximal convoluted tubule cells and decreased in the lumen and in the cells of the collecting and distal convoluted tubules. Other tissues, that is, epithelia, endocrine and exocrine glands, nervous system, cartilage and bone, and connective tissue of the skin and mucosæ, showed no fluorescence.

In order to determine the specificity of the above localizations of hormone, labelled Armour thyroid stimulating hormone inactivated by heating in acetic acid medium, was injected into rats in similar conditions. With the exceptions of the thyroid glands, the connective tissue proper and the adipose tissue and mast cells, which showed a weaker fluorescence, the rest of the tissues examined showed more intense fluorescence in the animals injected with inactivated labelled hormone. As opposed to the rats injected with active hormone where no fluorescence



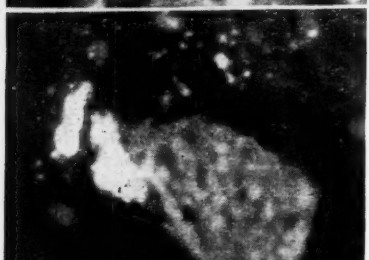


Fig. 1. Above, thyroid gland five minutes after the injection of labelled thyroid stimulating hormone. Fluorescence is present in the basal membranes of the follicles (×c. 1,100)
Fig. 2. Below, loose connective tissue thirty minutes after the injection of labelled thyroid stimulating hormone. Two mast cells, showing fluorescence in their granules, appear in the vicinity of an arteriole (×c. 2,130)

was observed in the small intestine, marked fluorescence was observed in the capillaries, in the epithelium and in the lumen of the small intestine in rats injected with inactivated hormone.

The advantage of this method appears to consist in that the labelled thyroid stimulating hormone partially retains its biological activity and provides new data on the localization and dynamics of the hormone at histological level. It also permits the control of the specificity of the hormone localization because of the difference in localization as compared with other labelled hormones and proteins[6].

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Maintenance of Parathyroid Tissue Fragments on Agar Substrates

Explantation of organ fragments on to plasma clots[1] or soft agar[2] contained in Maximow slides or embryological watch glasses permits the preservation and development of organotypic tissue *in vitro*. The recent finding that mammalian cell strains can be cultivated for extended periods with minimal effort on nutrient media solidified with agar[3,4] suggested the use of agar substrates to simplify and improve maintenance of functional organ fragments prior to surgical transplanatation.

Normal dog and human adenomatous parathyroid tissues were placed in calf or autologous serum 10 per cent in Hanks' balanced salt solution containing antibiotics, and cut into fragments 1 mm. in diameter or less. 4–6 such pieces were deposited on the surface of each of a series of agar slants[3]. The agar slants (3 ml. of solidified medium + 0·5 ml. of liquid medium) consisted of autologous serum 25 per cent, Eagle's supplements in balanced salt solution, antibiotics, and Difco 'Noble' agar 2 per cent. Nutrient materials reached explants by diffusion through the agar and provided adequate nutrition for at least a week. In one experiment, 3–4 fragments of human parathyroid adenoma were explanted to each of several Maximow slides containing weak plasma clots. Maximow slide cultures were handled as described by Escamilla *et al.*[5] The 0·5 ml. plasma clot systems used for primary explantation and for the 1st transfers contained autologous serum 10 per cent, donor plasma 15 per cent, balanced salt solution with antibiotics 65 per cent, and 1 drop of a thrombin-balanced salt solution mixture (50 units of thrombin per ml.). For subsequent transfers, individual fragments were placed in hanging drops of medium without thrombin. Clot liquefaction and/or lowering of *p*H by the explanted tissue necessitated transfer at 2–3 day intervals. Prior to cultivation, 1 fragment from each gland was fixed and processed for subsequent histologic examination.

Cultures were incubated at 34° C. and assessment of their quality was done by: (*a*) noting clot liquefaction and/or *p*H change; (*b*) determination of percentage of cells viable by means of eosin[6]; (*c*) comparison of histologic integrity before and after cultivation; and (*d*) in the case of human adenoma fragments, by evaluating the ability of agar cultivated fragments to cause alteration in serum calcium levels following transplantation to hypoparathyroid patients.

In agar slants, adult dog parathyroid fragments caused slow reduction of *p*H from 7·4 to 7·0 during 7 days of incubation. Renewal of the fluid phase of such cultures at 7-day intervals allowed their maintenance for longer than 3 weeks. 60–70 per cent of the cells remained viable throughout this period.

Fig. 1, *A–D*, shows the histologic appearance of dog parathyroid before and after 8 days on agar substrate. Prior to cultivation, the gland was predominantly composed of chief cells, organized in trabeculæ. After cultivation on agar the tissue maintained good cohesion of cells, although the trabecular pattern had diminished. The individual cells became elongated, in contrast to their normal polyhedral appearance, and many nuclei were seen to be oval or pyknotic. However, the endothelial linings of blood vessels were preserved, and a normal chromatin pattern was detectable in many of the chief cells.

Although slide cultures of human parathyroid adenoma were renewed at frequent intervals, *p*H fell to below 6·8 between the sixth and ninth day of cultivation. Subsequent transfer failed to reveal metabolic activity. Less than 3 per cent of the cells were viable at the ninth day, and histological examination showed marked necrosis of the tissue. These fragments were not used for transplantation.

The maintenance of human adenomatous parathyroid on agar obviated the need for renewal of medium or transfer of fragments since such cultures were not affected by acidity and depletion of nutriments. When terminated after 11 days *in vitro*, agar cultures contained about 65 per cent viable cells.

Fig. 1, *E–H*, presents the comparison of an adenoma before and after 11 days on agar. Prior to cultivation, the adenoma demonstrated large chief cells, oxyphils and water-clear cells. After 11 days *in vitro* the architecture of the tissue was looser, and the cells were larger and less eosinophilic. Giant nuclei persisted, and many of the cells contained the vacuoles which are thought to be associated with active function[7].

The fragments of agar-cultured adenoma were transplanted into a 16-year-old patient who had exhibited severe symptoms of hypoparathyroidism for the preceding 26 months[8]. Withholding of vitamin D therapy prior to transplantation resulted in a fall of serum calcium to 7·25 mgm. per cent. Fig. 2 shows that serum calcium was elevated to normal levels immediately after implantation of 40 fragments of agar-cultured adenoma. Similar results were obtained following implantation of agar cultured fragments to two additional recipients.

While the decline of serum calcium to pre-operative levels after 3 weeks is thought to reflect the recipient's immunologic response to the transplanted material, the initial elevation of calcium levels demonstrates that the tissue maintained on agar retained both viability and physiological function.

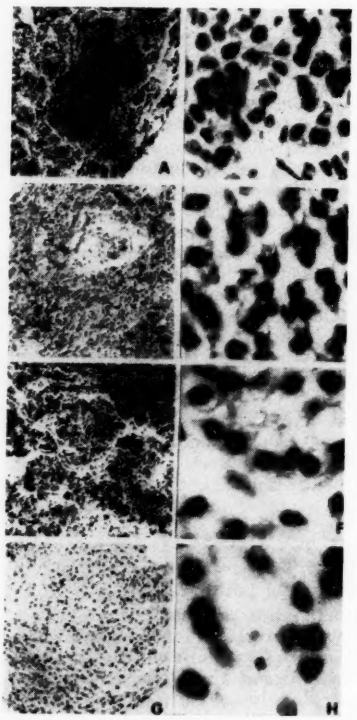

Fig. 1. Histological appearance of dog parathyroid and human parathyroid adenoma before and after cultivation on agar. (× 30 and × 270). *A* and *B*, dog parathyroid before cultivation; *C* and *D*, dog parathyrod after 8 days on agar; *E* and *F*, human adenoma before cultivation; *G* and *H*, human adenoma after 11 days on agar.

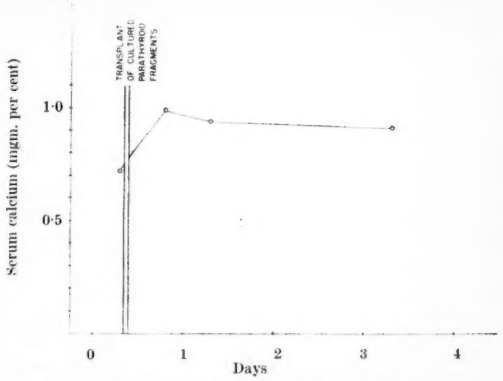

Fig. 2. Serum calcium level following transplantation of adenoma fragments cultured 11 days on agar

Since the use of solid nutrient substrates minimizes the care required to preserve specific functional elements in tissue fragments, further exploitation of agar systems for organ culture or for maintenance prior to transplantation seems desirable.

We wish to thank Dr. Francis D. Moore and Dr. Daniel Bernstein for providing the adenomatous tissue, Dr. Richard Wilson for serum calcium determinations, and Dr. John H. Hanks for helpful discussions throughout the work.

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BIOLOGY

A Method for the Quantitative Study of the Surface Precipitation Reaction

FOR almost as long as protoplasm has been recognized, some of its basic properties have been studied. Among these is the 'self-sealing' property or surface precipitation reaction. When a cell is broken, its contents do not diffuse into the surrounding medium but instead form discrete droplets. This reaction has been observed in many types of cells, but there has been little attempt to study it quantitatively. It would be highly desirable to know the amount of force required to break a cell; for if too much force is applied, the surface precipitation reaction may not be observed, since the cell is broken too quickly (for a discussion of the reaction, see refs. [1], [3]).

Cole in 1932[2] studied the surface properties of the sea-urchin egg by compressing it with a gold fibre. Because he was interested in the elastic properties of

the egg membrane and not in the surface precipitation reaction, he did not break the eggs. However, the apparatus he used is of interest, as it offers a possible method for studying the force necessary to break cells. The apparatus has one drawback for this purpose. When the egg is compressed by the gold fibre, it is also rolled between the fibre and the supporting surface, with the result that it is more or less 'smeared' rather than broken directly.

In an effort to overcome these difficulties, I devised an apparatus by modifying a DeNouy tensiometer so that it would apply a downward force instead of an upward one. The tensiometer, built by Cenco, uses a fine wire in torsion, with a scale at one end to indicate how much it is twisted. Attached to the centre of the wire is an arm which is used to apply the pressure. The apparatus was calibrated by using a microbalance and was found to be accurate to plus or minus 0·25 mgm. Attached to the end of the arm is a small glass plate, 5 mm. on each side, which is pivoted so that it will automatically level itself when brought down on a flat surface. In operation a single sea-urchin egg, amœba, or other cell is placed on a slide under a microscope, and the glass plate is lowered until it just touches the cell. A 'zero' reading is noted on the scale of the tensiometer. Additional force is then applied until the cell breaks. Then a second reading is taken, and the difference of the readings indicates the pressure applied.

Certain corrections have to be made before the reading can be considered indicative of the pressure required to break the cell. First, it was found that a certain force was required to displace the water under the glass when moving it down the 70 microns, more or less, required to break a sea-urchin egg. Secondly, the plate was not made as precisely as would have been desirable, and hence tends to become poorly aligned with respect to the slide. This factor is not easily accounted for and reduces the accuracy of the apparatus for cells as small as sea-urchin eggs. To correct for this inaccuracy the glass plate should be mounted in gimbals, as a ship's compass is mounted, rather than pivoted on one axis only, as it now is. For larger cells, such as the amœba *Chaos chaos*, this error becomes less important. Results of experiments with *Amoeba* have been published elsewhere (Heilbrunn *et al.*[4]).

The apparatus has one important use despite its inaccuracies. It can be used to break cells in order to study the surface precipitation reaction, since it offers a means of controlling the force used. Otherwise the reaction may not be properly observed.

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Limb Development in the Absence of Ectodermal Ridge

This communication is designed to indicate the current status of research concerned with the importance of ectoderm in limb morphogenesis in the chick embryo. Previous studies[1,2,3] indicate that the apical ectodermal ridge serves as an inductor of limb outgrowth, and that elaboration of distal structures ceases when it is removed. The experiments which provide the evidence for the importance of the apical ectodermal ridge are based on surgical procedures[1] and on techniques which involve the use of a chelating compound for preparing ectoderm-free mesoderm and trypsin for preparing mesoderm-free ectoderm[2]. Recently Bell[4] has postulated that the ectoderm may not be necessary for limb elaboration, a view which has also been taken by Amprino and Camosso[5]. In Bell's experiments, 19 per cent of limb bud stripped of ectoderm by means of focused ultrasound and grafted to the flank or cœlom formed skeletal parts representative of all proximo-distal levels of the limb.

Bell's observations provoked a renewal of interest in the fate of ectoderm-free limb mesoblast and in the possibility that distal development (formation of hand or foot digits) may occur in the absence of ectoderm. It soon became evident, as shown here, that there was not complete uniformity of results obtained with versene-stripped limbs, and it was agreed by us that it would be most sensible to join forces in a combined attack on the problem so that differences in technique, etc., could be resolved. Some preliminary experiments have already been carried out jointly. The following results, obtained in the individual laboratories, are presented as a prelude to those of the joint effort.

The Bell group (C. Merrill and R. Delson) has studied a series of 133 flank and intracœlomic grafts of limb buds from which ectoderm was removed by means of treatment with versene (ethylene-diaminetetracetic acid, disodium salt, 1 mgm. per cent in calcium- and magnesium-free tyrodes) for 30 min. Limb buds were at stage 19 at the time of treatment. In one experiment 19 per cent and in another 24 per cent of limb buds so treated formed distal structures.

Saunders and Gasseling have observed 100 chorio-allantoic grafts of versene-stripped buds (stages 18–21) and 31 grafts of controls; the latter were treated with versene for 60 min. and then grafted with the macerated ectoderm still in place. The stripped limb buds may be divided into two series: one exposed to versene for 30–40 min. and the other for 60 min. before mechanical removal of the ectoderm. These investigators observed a residual 'shiny' or 'refractile' layer which was difficult to separate from the mesoblast following removal of the ectoderm after the shorter exposure to versene. (This refractile layer has been observed by the other investigators, too.) Of 43 limb buds grafted after 30–40 min. of versene treatment, 4 developed definite terminal parts, 3 possibly had some terminal parts (phalanges) and 36 definitely showed no distal structures. Fifty-seven limb buds were treated with versene for 60 min. before removing the ectoderm. The refractile layer was readily eliminated following this treatment and none of these grafts developed distal limb elements. Twenty-five of 31 controls which had been treated with versene for 60 min. (but not stripped) developed distal limb structures.

Zwilling's cases consisted of 67 grafts (33 flank, 34 chorioallantoic) of limb buds which had been stripped of ectoderm following 30–40 min. of versene treatment. Under the conditions in his laboratory this treatment eliminated the refractile layer. Donor embryos were in stages 17–20, the majority at stages 18 and 19. None of these developed distal structures. Thirteen of 17 controls (exposed to versene for 30–40 min., but not stripped) developed distal structures.

The above results as well as results of the preliminary joint research show inconsistencies following removal of limb-bud ectoderm by means of versene, and these inconsistencies centre chiefly around the presence or absence of the refractile layer on the treated mesoblast. It has been observed that the refractile layer may cover all or part of the mesoblast and not necessarily only that portion which underlies the ridge. Grafts which develop distal parts appear to come from limb buds which have been treated in such a way that the refractile layer may be preserved; but how much is required and precisely where it must be located has not yet been determined. Evidence is not yet sufficient to decide whether the refractile layer is cellular or acellular; in the latter case, whether it may consist of basement membrane, intercellular matrix or both. It seems, therefore, that limb development may proceed in the absence of an apical ectodermal ridge as a morphological entity and that distal outgrowth in some experiments depends on the presence of a 'refractile' layer which may be non-cellular.

The observations described above do not invalidate the earlier experiments on the role of the ectoderm in limb development. For example, surgical removal of the ectodermal ridge definitely produces limbs which lack distal structures. This was first described by Saunders, has been confirmed by Amprino and Camosso[6], by Zwilling in unreported experiments and by others. The earlier findings must now be evaluated in the context of recent observations.

The authors plan future joint and independent investigations which will be designed to elucidate the nature of the refractile layer as well as its possible role in limb development.

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Glaucoma Species in the Central Nervous System of the Carp

THE free-living ciliate *Glaucoma pyriformis*, or at least a close relation, has been found to occur as a facultative parasite both in arthropods[1-6] and in the central nervous system of fishes[7] and of amphibians[8,9]. This parasitism may be rendered possible by the fact that this protozoan parasite is quite tolerant of carbon dioxide[10].

Recently we observed a *Glaucoma* infection in young larvæ of the carp *Cyprinus carpio*, in which about 8 per cent was found to be infected. The parasites were present in the cerebrospinal fluid, the brain, the spinal cord and the meningeal fluid.

On the strength of the morphological character the parasite could with certainty be identified as belonging to the genus *Glaucoma*: flask-like body with a sub-cylindrical anterior and tapering gradually to a rounded extremity, approximately 20 longitudinal rows of cilia with large basal bodies in the ectoplasm, wide cytostoma surrounded by larger cilia and containing two undulating membranes, large cytopharynx, single spherical macronucleus and very minute micronucleus, both situated in a separate clear area.

The average dimensions of the parasites were: length 59 μ, breadth 24 μ, depth 16 μ. The average diameter of the rounded parasites, which were found in the tissues of the brain and of the spinal cord, was 30 μ. Cyst formation was not observed.

Just as in the former cases of *Glaucoma* infection in fish[7] and amphibians[8,9] the infection probably took place after hatching. Subsequently the parasites could find their way from the skin of the intestine to the tissues of the central nervous system.

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Value of a Simple Observational Technique for Determining the Fat Content of Algal Cells

DURING the period 1953–55 an investigation was made of the survival of freshwater algæ during dry periods[1]. Some attention was paid to the accumulation of fat in the cells of diatoms and other algæ though details of this work have not yet been published. The method used to investigate fat accumulation was a simple one in which cells were measured in length and in width in girdle view. For some cells the width in valve view was also measured. Fat globules, stained with sudan black, were measured and a note made of the number of fat globules within each particular size range, present in each cell. On the basis of these results, determinations could be made, for comparative purposes, of the mean relative amount of fat per cell in sample populations. The results suggested that fat accumulation was invariably associated with drying in those species investigated which included *Pinnularia viridis*, *P. appendiculata*, *Nitzschia palea*, *N. frustulum* in addition to *Microspora stagnorum*.

Since late 1957 attempts have been made to check the accuracy of the observational technique by biochemical methods and to repeat some of the earlier work. From a sample of *Navicula pelliculosa* (Breb.) Hilse, kindly given to me by Dr. G. E. Fogg of University College, London, pure, unialgal cultures were set up. So far as possible, the method of culture described by Collyer and Fogg[2] was adopted. For most cultures, the medium used was Chu No. 10[3]. Determinations of dry weight, fatty acids and unsaponifiable lipoid were made in duplicate upon each sample by the methods described or referred to in the account by Collyer and Fogg[2].

Some determinations were made on material which had been withdrawn from liquid culture by centrifuging and then allowed to dry in air. The observational technique was used on parallel samples ($n=40$) which were all determined in triplicate. A summary of some of the results obtained (Table 1) indicated that although observation alone may sometimes give misleading results, this method usually gives a suggestion of the level of fat accumulation in cells. The small size of the cells (about 8×4 μ) makes this species one of the most difficult of the diatoms investigated in which to measure the diameters of the fat globules. It is possible that in dealing with larger cells (of *Pinnularia viridis*, for example), although the absolute error might be greater, the percentage error in observational results would be less. It has not been possible to confirm this as large diatoms have not, so far, despite many attempts, been grown in culture in sufficient bulk to allow of biochemical analyses. If fat determinations by observation alone do give some indication of the actual fat content of cells it may be concluded from earlier work that for certain algæ (for example, *Pinnularia viridis*) there may be an association between the position of the cell at the pond margin and the accumulation of fat in the cell (Table 2). Cells further away from the water contain more fat than cells near the water. It has also been found that the fat content of cells, as determined by observation, may be altered by drying artificially or by subjecting the cells to lower temperatures.

Table 1. Determinations of Fat in Cells of *Navicula pelliculosa* by Chemical Means and by Observation

Sample number	1	1.4	1*B*	2	3	4	5	6
Fat determined chemically, expressed as % dry weight.	6·0	8·3	8·9	24·1	5·4	8·6	11·5	14·1
Fat determined by observation, expressed as % fat per cell by volume.	5·7		11·4	15·0	9·6	8·1	48·9	4·7

Table 2. Determinations of Fat, by Observation, in Cells of *Pinnularia viridis* from a Litter Surface at Varying Distances from the Edge of a Small Pond in 1953 and 1954

	1953			1954				
Distance from pond edge (cm.)	5	15	100	5	5	15	60	100
Height above water level (cm.)	1	2·5	10	0·5	0·5	1	7·5	18
Numbers of cells per sample	4	6	7	23	20	15	25	4
Mean cell volume (to the nearest 1000 μ^3)	26	22	24	38	38	39	37	24
Mean volume of fat per cell (μ^3)	200	336	620	413	429	900	1234	1372
Mean % fat per cell (v/v)	0·8	1·5	2·5	1·1	1·1	2·3	3·4	5·7
P						0·01	0·05	0·001

If this simple observational technique proves, ultimately, to be sufficiently accurate, if only for comparative purposes, it presents three advantages over the use of biochemical techniques alone. It is quicker, does not require elaborate apparatus or more than a small quantity of algal material, and it involves observing individual cells. The combined use of both methods is being investigated further. The results so obtained might be used to test the conclusions of Kolbe[4] regarding stratified layers of oil and volutin in *Navicula oblonga*. The misleading result obtained by observation for sample 5 (Table 1) may have been due to the existence of hollow spheres of fat surrounding cores of some other substance (possibly volutin) in the cells of the diatom. That fat and some other substance may be formed close together in cells of at least one diatom, *Pinnularia*

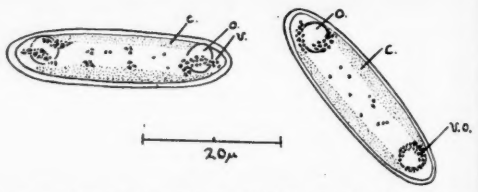

Fig. 1. Cells of *Pinnularia appendiculata* stained with methylene blue.
Left, cell showing granules, *v.*, which are assumed to be volutin, concentrated at the polar regions of the cell, near the fat globules, *o.*; right, cell showing, at one pole, granules almost completely surrounding a fat globule, *v.o.* *c* = chromatophore.

appendiculata, has been observed (Fig. 1). The small size of the granules of the other substance mean that the metachromasy exhibited with dilute methylene blue cannot be considered as evidence that the granules were volutin. In this instance, however, the granules were apparently being deposited on the outside of the fat globules. Such an occurrence might lead to an underestimate of the fat content by the observational technique and might explain the results obtained in sample 6 (Table 1) for *Navicula pelliculosa*.

Attempts to grow large diatoms in bulk are being continued, and I should be grateful to receive samples of healthy, freshwater diatoms of large size, accompanied by a brief description of the habitat.

My thanks are due to the Central Research Fund Committee for providing me with a grant to purchase a vacuum oven which was required for the biochemical analyses.

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Production of Fungal Arbuscular Organs on Laboratory Media

Interest in the morphology of fungal assimilatory organs was aroused by the appearance of vesicles and arbuscles on hyphæ of *Memnoniella echinata* penetrating textile fibres during cellulolysis. Vesicles have been reported on hyphæ within a medium when they are grown under conditions of deficient aeration, increased ionization, physiological unbalance of temperature, humidity or nutrition, and especially after the addition of bacterial, fungal or plant metabolites. No arbuscles have been obtained by these means, probably because the conditions or concentrations necessary for their appearance suppress germination of spores and growth of the fungus. The temporary arbuscular development on certain mutant strains of *Phytophthora cactorum* described by Stamps[1] suggests lack of a biosynthetic intermediate concerned with cell-wall structure. Inhibition of normal metabolic pathways, accentuation of overflow intermediates, and the functioning of latent shunt reactions, probably underlie vesicular development under the conditions enumerated above. The transformation of Gram-negative bacilli into swollen spherical cells because of reduction in cell-wall content of muramic and diaminopimelic acids described by Salton and Shafa[2] as the effect of a fungal metabolic product, and by Brenner *et al.*[3] as the result of lysozyme treatment,

bacteriophage action, excess lysine, and deprival of diaminopimelic acid, together with the recent work of Eddy and Williamson[4] on the development of irregularly shaped outgrowths on yeast protoplasts under the action of digestive juices, seem to be similar phenomena. In the experiments now to be described, I accept as a working hypothesis that vesicles and arbuscles are primarily a manifestation of fungitoxic factors, though an element of adaptation to nutritional requirements undoubtedly enters into their morphology.

A technique was developed which permitted germination and early growth of the fungus on a normal medium but favoured rapid accumulation of metabolic products around the hyphæ and partial anærobic conditions. A double-dish method was used, the outer dish containing the medium, while the inner dish had a base of nylon fabric, superimposed upon which was the aerial part of the fungus colony. The apertures between the nylon threads permitted immediate access to about 4 per cent of the surface of the medium. These conditions in themselves were sufficient to produce vesicles within the more complex organic media, which, within a few days, contained clusters of hyphæ with swellings at the tips, the vesicles differing in shape and size according to the organism being grown and the nature of the medium. Vesicles were rare in mineral salts/sucrose medium, but were numerous and multiple in malt agar to which 'Vegemite' (autolysed yeast) was added. By lifting out the inner dish with its superimposed colony, thus breaking off the hyphæ passing through the fabric base, the outer dish containing the medium could be examined, and anti-fungal or other compounds inserted, or the *p*H or moisture content changed. The basal hyphæ remaining within the medium could be distinguished from the new growth which occurred when the inner dish was replaced. Replacement of the established colony on a medium rendered toxic by metabolic products, especially of certain other fungi, by low concentrations of fungicidal compounds, or by a physiologically unbalanced nutrition, led to arbuscular development on the new growth of assimilatory hyphæ, and to coralloid masses of plectenchyma when water was removed from the medium. The proliferated hyphæ spread in far more complicated patterns than those figured by Stamps[1], and were usually mixed with coils, loops and swollen outgrowths.

Organisms used in the experiments represented strains with varying tolerance to the toxic additives in the medium, and ranged from *Aspergillus niger*, through the cellulolytic species *Memnoniella echinata*, *Myrothecium verrucaria* and *Botrytis cinerea*, to *Penicillium rugulosum*, which is parasitic on fungi and was found to be extremely resistant to fungal metabolic products. It was not easy to obtain arbuscles on *P. rugulosum*. Interesting results followed when one species was transferred to the medium from which another had been removed. The successful biotroph was found to leave a medium relatively atoxic but to be tolerant to metabolites; but the necrotroph excreted products which caused changes both in the vegetative and reproductive structures of its own or other species. Tolerance of certain strains of fungi to fungitoxic substances was correlated with lessened arbuscular development.

I believe this work is linked with the general problem of haustorial organs formed by parasitic fungi inside host plants, and suggest that the technique may be of value to plant pathologists interested in exploring host and parasite interactions.

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Hybrids of *Tilapia nilotica* × *T. galilea*

In a recent communication, C. F. Hickling reports on hybrids between Malayan *Tilapia mossambica* stock and an East African one. It may be of interest, therefore, to record our observations, which indicate that hybridization among this genus might be more common than it is generally believed.

In summer 1958 a small mud pond at the Fish Culture Research Station, Dor, was stocked with several *Tilapia nilotica* males and *T. galilea* females. When this pond was drained, about 70 small *Tilapia* fry were discovered among the weeds. These fry were believed to be the hybrids of the two species of *Tilapia* stocked.

In order to ascertain this, we stocked again in the spring of 1959 two mud ponds and several concrete tanks with carefully sexed males of *T. nilotica* and females of *T. galilea* and vice versa.

In May and June courtship of males and females of these species, and also nest building in the mud ponds were observed. In the middle of June, small fry, the offsprings of *T. nilotica* (male) and *T. galilea* (female) were seen in one of the concrete ponds.

Only further work in this direction will show the importance of the *Tilapia* hybrids for fish culture.

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ENTOMOLOGY

Contents of Cystine-Cysteine, Glutathione and Total Free Sulphydryl in Arsenic-resistant and sensitive Strains of the Blue Tick, *Boophilus decoloratus*

The affinity of arsenicals for sulphydryl groups was first suggested by Ehrlich as being responsible for the toxic action of these poisons, and the work by Voegtlin[1] and his associates later demonstrated that the trypanocidal action of arsenicals was reversed as well as prevented by the administration of reduced glutathione, cysteine and related sulphydryl compounds.

In insects, Fink[2] and Forgash[3] have shown that significant drops in reduced glutathione levels occur after injection of arsenious oxide. In addition, the latter found that glutathione, when injected into the cockroach, protected it against the toxic effects of arsenic. Harington[4] has shown that the levels of free sulphydryl and reduced glutathione in arsenic-resistant larval strains of the blue tick, *B. decoloratus* are considerably depressed following treatment of the larvæ with varying concentrations of arsenious oxide.

It was suspected, therefore, that higher levels of sulphydryl compounds might be found in resistant larval ticks, so conferring upon them a greater power to detoxicate a given amount of arsenious oxide than is possessed by sensitive larvæ, and thus contributing towards a greater resistance to arsenic. In the present work, the levels of cystine, cysteine, reduced glutathione and of total free sulphydryl in both strains of larvæ were determined after deproteinization of the material with 3 per cent sulphosalicylic acid. Cystine and cysteine were determined by the method of Park and Speakman[5] using phospho-18-tungstic acid, reduced glutathione by the glyoxylase-manometric method of Woodward[6], and total free sulphydryl by the ferricyanide method described in Snell and Snell[7]. Two series of determinations for cystine and cysteine were made at an interval of 3 months, using fresh larvæ in each case. The present work is an extension of that of Thompson and Johnston[8] who found that the level of sulphydryl in resistant tick eggs at a stage midway between oviposition and hatching was approximately twice that in the sensitive strain.

The results of the present investigations are given in Tables 1–3.

The first series of determinations was carried out on such a small sample of the tick population that no testing for departure from randomness was possible. For the second series the 95 per cent confidence range of the difference of 1·82 mgm. cystine/gm. tick material is ±0·56 mgm./gm. so that the difference is real. The level of cystine showed an increase in series 2 over series 1, while no cysteine was detected at all in the second series. This suggests that the quantities of cystine and cysteine and possibly their ratio may vary with time during the life of the larvæ.

Analysis of the above data shows that the difference between the means obtained is significant at the 1 per cent level and that the 95 per cent confidence limit differences between the *R* and *S* means are 175·7 and 341·7 respectively.

The difference between these means is significant at the 1 per cent level. The 95 per cent confidence limits of the difference in total free sulphydryl content of the *R* and *S* strains are 18·3 and 55·9 respectively.

I wish to thank Mr. D. C. Goch for the statistical examination of the results, Mr. G. B. Whitehead for helpful discussions and Mrs. X. Proimos for the experimental work carried out.

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Table 1. Cystine and Cysteine Contents of Resistant and Sensitive Larval Ticks expressed as mgm./gm. Tick Material

Material	No. of duplicate determinations	Resistant strain	No. of duplicate determinations	Sensitive strain	Difference *R*—*S*	95% confidence limits of difference
First series						
Cystine	7	1·46	5	0·23	1·23	No analysis possible
Cysteine	7	0·67	7	0·19	0·48	
Second series						
Cystine	8	2·81	10	0·99	1·82	±0·56
Cysteine	12	none detected	12	none detected		

Table 2. Reduced Glutathione Content of Resistant and Sensitive Larval Ticks expressed as μgm./gm. Tick Material

Material	No. of determinations	Resistant strain	No. of determinations	Sensitive strain	Difference *R*—*S*	95% confidence limits of difference
Reduced glutathione	20	427·1	10	168·4	258·7	±83

Table 3. Total Free Sulphydryl Content of Resistant and Sensitive Larval Ticks expressed as μgm. SH/gm. Tick Material

Material	No. of determinations	Resistant strain	No. of determinations	Sensitive strain	Difference *R*—*S*	95% confidence limits of difference
Total free sulphydryl	18	70·8	12	33·7	37·1	±18·8

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Resistance of Houseflies to γ-Benzene Hexachloride and Dieldrin

The following observations have been made during a study of the mechanisms of resistance to γ-benzene hexachloride in a strain of *Musca domestica* obtained initially through the kindness of Dr. J. R. Busvine. The flies (*S*-flies) had a history of dieldrin resistance[1]; but the first generation of adults at this Laboratory were found to be only slightly more resistant to dieldrin and γ-benzene hexachloride than the normal Laboratory culture of *Musca domestica*. On incorporation of graded amounts of dieldrin into the larval medium of the *S*-flies the level of resistance rose rapidly. After six generations a concentration of 150 p.p.m. of dieldrin per wet weight of larval medium had been attained, and this concentration has been maintained during subsequent generations. These flies (*R*-flies) were highly resistant to both dieldrin and γ-benzene hexachloride although at no time has the latter been used for selection at this Laboratory.

When eggs from *R*-flies were bred through a dieldrin-free larval medium, the emerging adult flies showed a decrease in their resistance to both dieldrin and γ-benzene hexachloride, the drop being more marked with the latter insecticide. This sub-strain was designated *RND* and further breeding in a dieldrin-free medium caused no further drop in their resistance. This drop in resistance was surprising as it was shown by a bio-assay technique that the adult *R*-flies contained considerable amounts of dieldrin in their tissues (Table 1) so that a lower dose of applied insecticide would be expected to produce the same toxic effect in the *R*- compared with the *RND*-flies.

Table 1. Amounts of Dieldrin Present in Adult Female *R*-flies determined by Bio-assay of Acetone Extracts on *S*-flies

Age of Adult (Days)	μgm. dieldrin/fly
1	8·4
2	6·9
3	6·9
8	4·8
15	1·8

The fact that the drop in resistance occurred only on the initial removal of the dieldrin pressure suggested that it was not controlled by any genetic factor as both groups of flies were progeny of the same parents. The effect was immediately reversible, for when eggs were taken from the *RND*-flies and bred through a dieldrin-containing medium, the level of resistance of the emerging adults rose to that of the *R*-flies.

Tests showed that there was no difference in the percentage emergence of the *R*- and *RND*-flies indicating that the dieldrin was not still selecting the more susceptible flies in the colony. For the purpose of these tests the standard housing and feeding of the flies[2] was slightly modified. The flies were allowed to emerge in 7 lb. glass jars instead of cages and only glucose and water was provided instead of the normal glucose, milk and water diet. It was found that the *R*-flies bred under these modified conditions had a lower level of resistance to γ-benzene hexachloride than had previously been observed for the *R*-strain but still higher than that of the *RND*-flies. When milk was re-introduced into the diet, the resistance of the *R*-flies returned to the higher level. A series of tests were carried out to confirm these observations, the results of which are summarized in Table 2. The LD_{50} values to γ-benzene hexachloride are given. These were estimated by observing the

Table 2. EFFECTS OF DIET AND DIELDRIN PRESSURE ON RESISTANCE OF ADULT HOUSEFLIES TO γ-BENZENE HEXACHLORIDE

	R-flies (Larvæ bred in medium containing 150 p.p.m. dieldrin)		*RND* flies (Larvæ derived from *R*-flies but bred in dieldrin free medium)	
	R_1-flies (Adults fed on milk, glucose and water)	R_2-flies Adults fed on glucose and water)	RND_1-flies (Adults fed on milk, glucose and water)	RND_2-flies (Adults fed on glucose and water)
LD_{50} values μgm. γ-benzene hexachloride fly — Male	5·9	1·3	1·2	0·8
LD_{50} values μgm. γ-benzene hexachloride fly — Female	16·9	4·3	2·2	1·7

20-hr. knock-down figures for a range of concentrations. The flies, which were bred under identical conditions apart from difference in diet, were treated with 2 μl. of acetone solution of γ-benzene hexachloride applied topically to the dorsal surface of the thorax kept at 25°C. and provided with glucose and water only during the toxicity test. The age of the flies varied between 4 and 9 days.

The LD_{50} values for the R_1-flies are 3·9 (female) and 4·5 (male) times those for the R_2-flies whereas the values for the RND_1-flies are only 1·3 (female) and 1·5 (male) times the values for the RND_2-flies. The experiments of Table 2 were repeated in which the milk was replaced with casein, milk cream or fat extracted from milk cream with petroleum ether (boiling range 40–60°C.). The results are given in Table 3.

Table 3. EFFECT OF SUBSTITUTING CASEIN, CREAM OR 'MILK FAT' FOR MILK IN THE DIET OF *R*- AND *RND*- ADULT FLIES

LD_{50} values (μgm. γ-benzene hexachloride/fly)

Substituent for milk in R_1- and RND_1-flies		R_1	R_2	RND_1	RND_2
Casein	Male	1·3		2·0	
	Female	2·1		3·8	
Cream	Male	5·1	1·5	1·2	0·5
	Female	12·5	5·1	1·5	1·8
'Milk fat'	Male	5·5	2·0	1·3	0·7
	Female	16·3	6·7	3·2	1·8

The replacement of milk with cream or 'milk fat' but not casein gave LD_{50} figures of a similar order to those obtained when milk was available.

These results suggest that the presence of dieldrin in the larval food or adult tissue results in a higher level of resistance to γ-benzene hexachloride of the adult fly which receives dietary milk fat or some associated fraction. Moreover this property does not appear to be transmitted genetically in the absence of dieldrin pressure. This higher resistance is not merely due to an increase in the fat content of the insect itself as no significant difference was found between the total fat contents of the *R*-, *RND*- and *S*-flies.

Metabolism of benzene hexachloride labelled with carbon-14 by female *R*-, *RND*- and S-flies has been studied using the non-toxic α-isomer as an indicator of any differences in the rates of metabolism of the γ-isomer (c.f. Bradbury and Standen[3] and Oppenoorth[4]). It has been found that the *R*-flies metabolize benzene hexachloride at the same rate irrespective of whether they have been fed on glucose alone or glucose and milk but rather more rapidly than the *RND*-flies. The *S*-flies metabolize the α-benzene hexachloride at a rate intermediate between the two resistant groups. It is unlikely therefore that an enhanced rate of metabolism of the insecticide is responsible for any large part of the resistance to γ-benzene hexachloride of this strain of flies.

From these observations it is possible to divide the resistance to γ-benzene hexachloride of these flies into three levels :

(1) The difference in resistance between the *RND*- and *S*-flies which seems to be the major resistance likely to be found in the field and is not due to the *RND*-flies ability to metabolize the insecticide at an increased rate.

(2) The difference in resistance between the R_2- and *RND*-flies which is possibly due to the R_2-flies enhanced ability to metabolize the insecticide to non-toxic metabolites.

(3) The difference in resistance between the R_1- and R_2-groups of flies which is associated with dietary fat at the adult stage.

The last two differences only occur when the fly is under dieldrin pressure.

Fuller details of these experiments will be published elsewhere. The authors are indebted to Mrs. A. Brooks for her work in maintaining fly cultures. The experiments are part of an investigation into the mechanisms of dieldrin resistance in Diptera, supported in part by the World Health Organization.

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Dormancy of the Khapra Beetle: Quiescence or Diapause

THE khapra beetle, *Trogoderma granarium* Everts, is a dermestid pest of stored grain from India, now established in British malt stores. Ecological work in these stores and laboratory experiments have revealed a very interesting life-cycle.

After entering bulks of malted barley in malthouses, the insect rapidly passes through many generations, each of which is completed in about a month in the best conditions. When fæcal pellets accumulate in regions of the bulks where the temperature falls a little, some of the large larvæ delay pupation, leave the food and cluster in crevices in the fabric of the stores. There they remain for many months in a dormant condition with a very low rate of respiration. A variable proportion of these larvæ emerge from the dormant condition and pupate (*a*) after new malt has been provided, or (*b*) after a fall in temperature below 21°C. for a month, followed by a return to a high temperature, or (*c*) after a substantial increase in temperature within the breeding range, 21–40°C. In case (*c*), the greater the temperature increment, the greater is the pro-

portion pupating, particularly when the final temperature is within the upper part of the range. Such changes in temperature commonly occur during the loading and unloading of a store. At loading, the dormant larvæ emerge, pupate and breed rapidly in the new warm malt, producing both short-lived and dormant larvæ, thus completing the population cycle. Another interesting feature is that occasional feeding periods and moults occur during the dormancy.

This dormancy shows similarities to both quiescence and diapause. Quiescence is an arrest of development caused by unfavourable environmental conditions and ended as soon as favourable conditions return. Diapause continues in conditions suitable for growth until the animal is subjected to certain well-defined environmental changes, often not themselves allowing growth, but permitting some physiological change which enables growth to be resumed as soon as conditions favour it.

The dormancy of *T. granarium* resembles quiescence when it is terminated by fresh food or by a direct increase of temperature within the breeding range. However, not all the larvæ resume development and pupate in fresh food; indeed, many remain dormant even if the food is renewed continually. Also dormancy lasts many months, and sometimes years, if the temperature is held at 30°C., which cannot be regarded as unfavourable for growth: the larval period of insects not entering dormancy is only 6 days longer at 30°C. than at the optimum (35–40°C.); when the dormant insects are first held for a long enough period at a low temperature, they pupate without delay at 30°C. Thus this dormancy appears to be more complicated than quiescence.

For example, it is maintained at temperatures well within the breeding range, and in temperatures fluctuating daily (for example, 25⇌40°C.), also it is ended by exposure to conditions too cool for breeding. In diapause of this nature, it is generally postulated that the low temperature permits some physiological change, which is a prerequisite for the continuation of development on return to the higher temperature, and that this change takes place only slowly or not at all at the higher temperature[1]. However, the pupation of the dormant khapra larva may promptly follow an increase within the breeding range, for example, 30–35°C. This is unusual. Presumably the increase acts as a stimulus upon certain physiological processes, as apparently in those species whose diapause is broken by singeing, chemicals and other shock treatments. It appears, therefore, that this dormancy is a weak diapause, easily broken in various ways. It is facultative in that not all individuals enter diapause.

The diapause is well adapted to the environment in malt stores. It serves as a timing mechanism ensuring the simultaneous maturation of the sexes and probably also their survival in unfavourable conditions. In malt stores favourable temperatures usually occur when new malt is loaded, with little relationship to the seasons. A diapause rigidly geared to seasons would often prevent breeding in malt stores, but the breaking of diapause at high temperatures permits almost immediate breeding. The diapause is not broken by daily alternation between a low temperature, for example, 21°C., and a high one, for example, 35°C., either of which alone would break it. This avoids metamorphosis in empty stores where such alternations are common and in which there is almost no food. A quiescence would be less well adapted because it would permit metamorphosis in empty stores.

The original ecological basis of this diapause may be explicable when the insect has been studied in its natural habitat, probably in India. Limited studies have suggested that other species of *Trogoderma*, less common in food stores, have a diapause more difficult to break than that of *T. granarium*.

A full account of this work will be published elsewhere.

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BACTERIOLOGY

Amino-Acids of Red Sulphur Bacteria

The growth of autotropic unicellular organisms in purely inorganic medium is of great interest in biological studies. The production of amino-acids by such organisms is intimately connected with this growth. In the case of unicellular green algæ, the production of amino-acids have been investigated by Fowden[1], Pearsall and Fogg[2] and Eny[3]. I have attempted to determine the free and bound amino-acids produced in the photosynthetic red sulphur bacteria using the technique of paper chromatography. These experiments also furnish a comparative study of the protein units of algæ and photosynthetic bacteria.

The bacteria were grown from a mud sample supplied by Dr. Gaffron. The growing medium was that used by Hendley[4] and it consisted of a solution containing (per cent): 0·5 sodium chloride, 0·1 ammonium chloride, 0·05 potassium dihydrogen phosphate, 0·63 anhydrous sodium carbonate, 0·3 sodium thiosulphate ($Na_2S_2O_3$, SH_2O), 0·01 disodium salt of ethylenediamintetraacetic acid.

Trace elements such as those used by Larson[5] were also added in the above medium and 0·01 per cent sodium sulphide (Na_2S, $9H_20$) was added before inoculation to remove the residual oxygen in the medium. 'Pyrex'-distilled water was used all through the experiments; *p*H was adjusted by dilute hydrochloric acid to 8·2. Temperature was maintained at 35°C. and a light intensity of 170 ft.-candles was used for the growth of bacteria inside the incubator. Van Neil's[6] closed-bottle technique was followed and the bacteria was grown for 20–24 days.

In extracting the amino-acids from the bacteria the method followed by Fowden[1] was used. After harvesting, the bacteria were washed, killed and dried at laboratory temperature. The uncombined amino-acids were extracted from the dried, finely ground materials with 70 per cent alcohol at laboratory temperature for 15 hr. 1 c.c. of the aqueous alcohol was used for each 4 mgm. of material. The insoluble residue was then hydrolysed for 24 hr. at 105°C. with 6 *N* hydrochloric acid. The alcohol extracts and protein hydrolysates were concentrated and then desalted[7] using 'Nalcite SAR' (60–100 mesh) and finally evaporated to dryness.

The amino-acids present in these preparations were determined using two-dimensional paper chromatography. Aliquots containing material corresponding to 50 μgm. of nitrogen as estimated by the micro-Kjeldahl method were used for each chromatogram. Water-saturated phenol in the presence of ammonia vapour (Fowden[1]) was used as the first developing solvent and the *n*-butanol/acetic acid mixture of Partridge[8] was the second solvent. Table 1 shows the relative intensities of the amino-acid spots obtained after treatment with ninhydrin of the developed chromatograms.

Table 1. AMINO-ACIDS OF RED SULPHUR BACTERIA

Amino-acid	Free	Combined
Glycine	+	+++
Serine	+	−
Cystine	+	++
Methionine	+	+
Cysteic acid	−	+++
Aspartic acid	−	+++
Cysteine	−	++
Taurine	−	++
Hydroxyproline	−	++
Valine	−	+
α-Alanine	−	+++
γ-Aminobutyric acid	−	+++
Tryptophan	−	+++

+++, Strong ninhydrin colour ; ++, moderately strong ninhydrin colour ; +, Weak ninhydrin colour.

Comparing the results of Table 1 with those obtained by Fowden[1] in his work on the production of amino-acids by photosynthetic green algæ, it is seen that some of the important amino-acids which occur in algal proteins are also present in the proteins of red sulphur bacteria, but unlike the algæ, a few of the amino-acids occur in the free state in the bacterial proteins. Glycine occurs in appreciable quantities both as free and bound amino-acid in algal proteins, but in the case of red sulphur bacteria only traces of glycine are found in the alcoholic extract although an appreciable quantity of glycine occurs in the hydrolysate. γ-Aminobutyric acid occurs in appreciable quantities in the protein hydrolysate of red sulphur bacteria and in the free state in *Chlorella vulgaris* only. Glutamic acid and α-alanine are found in large quantities in the free state in all the algæ, but in the case of red sulphur bacteria only α-alanine is present in large quantities in the hydrolysate. Tryptophan occurs in large amounts in the hydrolysate of bacterial proteins but only in traces in algal proteins.

The important sulphur-containing amino-acids, namely, cystine, cysteine, methionine and cysteic acid figure prominently in the proteins of red sulphur bacteria ; but only cystine and methionine are found, in trace amounts, in algal proteins. Some of the essential amino-acids, namely, threonine, lysine, histidine, arginine, leucine, *iso*leucine are absent from the proteins of red sulphur bacteria. A smaller number of amino-acids are found in red sulphur bacteria than in algæ.

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A New Nitrogen-Fixing Bacterium

A NITROGEN-FIXING bacterium apparently different from all previously known organisms with this property was isolated from West Bengal soil by one of us (R.B.).

The bacterium appears after 20–24 hr. in nitrogen-deficient glucose or mannitol agar as big rods with rounded ends and homogeneous cytoplasm (measuring 3·5–6·0 × 1·0–1·2 μ) ; older cells vary in length and contain a row of refractile granules. Long filaments and swollen 'involution' forms arise in various media with combined nitrogen. The cells are Gram-negative and do not form endospores. Motility is sometimes seen but is quite erratic.

Cultures on nitrogen-deficient agar media show a characteristic uneven growth, at first thin and semi-transparent, later with isolated areas of heavy, raised growth that becomes coarsely wrinkled and changes from pale yellow to dark red-brown. The consistency is exceedingly tough and rubber-like, with age becoming more soft and smeary. Growth in sugar-free peptone media is scant, with a thin, tough but rapidly disintegrating pellicle in broth. Gelatine is not liquefied. Milk is slowly clarified and becomes semi-transparent and viscous, with alkaline reaction. Glucose, fructose, ethanol, glycerol and mannitol are readily utilized, mostly with acid formation. Xylose, galactose, di- and polysaccharides and numerous lower organic acids are not utilized. Growth occurs at *p*H about 5–9 and only under aerobic conditions. Nitrogen fixation in liquid media is slow but may result in gains of nitrogen exceeding 20 mgm. per gm. glucose or mannitol supplied.

Molybdenum appears to be essential for nitrogen fixation and cannot be replaced by vanadium. Fixed nitrogen (for example, ammonium and especially glutamic acid) allows a faster and more even growth than free nitrogen.

The organism differs markedly from Azotobacter, *inter alia* by its typical long rod-shaped cells which except for the negative Gram-reaction and the lack of endospores resemble those of Bacillus. On the other hand it resembles the genus Beijerinckia in the tenacious growth on agar but differs by its much larger cell size and the absence of typical polar fat globules. In our opinion this organism must be considered a separate group within the Azotobacteraceæ[1]. A more detailed description will be published elsewhere.

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Nicotinamidase in *Mycobacteria* : A Method for Distinguishing Bovine Type Tubercle Bacilli from other *Mycobacteria*

BOVINE type tubercle bacilli have been differentiated from other *Mycobacteria* by means of growth characteristics and pathogenecity for animals. They usually grow dysgonic in its original culture and are pathogenic for guinea pigs and rabbits. However, many laboratory strains of bovine tubercle bacilli now grow eugonic and can not be differentiated from growth characteristics, and furthermore, avirulent strains of bovine type tubercle bacilli like *BCG* or isoniazid resistant bovine tubercle bacilli have been developed, and they can not be differentiated from animal pathogenecity. Konno *et al.*[1,2] suggested simple chemical method to distinguish human tubercle bacilli from bovine tubercle bacilli or atypical *Mycobacteria* applying high nicotinic acid production of human tubercle bacilli. Halpern and Grossowicz[3] studied nicotinamidase activity of the extracts of *M. phlei* and *BCG* and reported *M. phlei* revealed high nicotinamidase activity than *BCG*. Bönicke and Lisboa[4] compared nicotinamidase of emulsion of human tubercle bacilli and bovine tubercle bacilli and

reported high nicotinamidase of human tubercle bacilli.

The present investigation aims at distinguishing bovine type tubercle bacilli from other *Mycobacteria* (human tubercle bacilli, bovine tubercle bacilli, atypical acid-fast bacilli and non-pathogenic acid-fast bacilli) by nicotinamidase activity. Fifteen strains of various types of *Mycobacteria* grown on the surface of Sauton's synthetic liquid medium for 3 weeks (one week for non-pathogenic acid-fast bacilli) were filtered off, washed three times with water. Cell-free extracts were prepared by grinding mechanically the washed bacteria in a cold mechanical mortar with half the amount of sea-sand for 30 min. Cell debris was removed by centrifuging in the Hitachi preparative ultra-centrifuge in the cold at 10,000 *g* for 40 min. Then the cell-free extracts were dialyzed overnight against distilled water at 6°. The protein content of the extracts was determined by Mehl's biuret method[5].

The bacterial extracts which contain 2 mgm. of protein served for the determination of nicotinamidase. Ten μmoles of nicotinamide in 0·05 *M* of phosphate buffer (*p*H 7·0) were added to each bacterial extract to reach a total volume of 2 ml. As a control, phosphate buffer with bacterial extracts without nicotinamide was used. They were placed in incubator at 37° for 3 hr. Ammonia formed in the system were distillated by Folin's method into *N*/40 sulphuric acid and then determined colorimetrically by Russel's phenol-hypochlorite method[6].

As shown in Table 1, when 10 μmoles of nicotinamide were used as a substrate, human tubercle bacilli produced 3·2–3·7 μmoles of ammonia, bovine tubercle bacilli were only 0·1 μmoles, avian tubercle bacilli were 3·8 μmoles, atypical acid-fast bacilli were 3·2–3·3 μmoles, and non-pathogenic acid-fast bacilli were 6·8–7·4 μmoles. Ammonia formation by bovine tubercle bacilli, either virulent or attenuated, was less than one thirtieth that of human, avian and atypical acid-fast bacilli, and about one sixtieth of non-pathogenic acid-fast bacilli.

For simplifying this procedure, qualitative method using bacterial emulsion was tried. About 100 mgm. of bacteria grown in Sauton's liquid medium was emulsified and centrifuged at 3,000 r.p.m. for 20 min. in physiological saline solution three times to remove ammonia from the surface of bacteria. Then, a bacterial emulsion of about 20 mgm. per ml. in physiological saline solution was made. Two test tubes containing each 1 ml. of emulsion served for the test. Three hundred μgm. of nicotinamide in 1 ml. of 0·02 *M* phosphate buffer (*p*H 7·0) was added to a test tube. Another control test tube contained bacterial emulsion in phosphate buffer without nicotinamide.

These test tubes were incubated at 37° for 12 hr. and ammonia produced in the test tube was determined qualitatively by Russel's phenol-hypochlorite method. Positive test showed green blue to deep blue colour development; on the other hand, the control was amber.

Forty strains of various kinds of mycobacteria were tested for nicotinamidase qualitatively. As shown in Table 2, only bovine tubercle bacilli gave a negative test except one questionable positive strain. All other *Mycobacteria* gave a positive test irrespective of virulence. On performing this test, it is recommended to wash out the ammonia on the surface of bacteria thoroughly before adding nicotinamide. If ammonia remains, it will disturb the test.

Table 1. Nicotinamidase Activity of Extracts from Various Mycobacteria

Types	Strains	Substrate Nicotinamide (moles)	Ammonia formed (moles)
Human tubercle bacilli	*H37Rv*	10	3·3
	H37Rv INH-R	10	3·2
	H37Ra	10	3·7
	RI Rv	10	3·6
Bovine tubercle bacilli	Ravenel	10	0·1
	Ravenel *INH-R*	10	0·1
	BCG (Japan)	10	0·1
	BCG (Copenhagen)	10	0·1
Avian tubercle bacilli	4110	10	3·8
	4121	10	3·8
Atypical acid-fast bacilli	*P* 8	10	3·3
	P 22	10	3·2
Non-pathogenic acid-fast bacilli	*M. phlei*	10	6·8
	M. 607	10	6·8
	M. smegmatis	10	7·4

*p*H 7·0: incubation at 37° for 3 hr.

Table 2. Qualitative Nicotinamidase Activity of Emulsion from Various Mycobacteria

Types	Strains	Nicotinamidase test positive	Nicotinamidase negative
Human tubercle bacilli	Virulent	10	0
	INH resistant	2	0
	attenuated	3	0
Bovine tubercle bacilli	Virulent	1 (±)	6
	attenuated	0	3
Avian tubercle bacilli		5	0
Atypical acid-fast bacilli		5	0
Non-pathogenic acid-fast bacilli		5	0
Total		31	9

*p*H 7·0, incubation at 37° for 12 hr. (substrate nicotinamide 300).
Positive nicotinamidase: green to blue colour
Negative nicotinamidase: amber colour

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Affinity of Lysozyme to Structural Elements of the Bacterial Cell as studied with Enzyme Labelled with Radioactive Iodine

When cells of *Bacillus megaterium* are suspended in a dilute phosphate buffer and lysozyme is added to this suspension, the cell walls are completely dissolved. After the removal of the protective walls, the structural integrity of the protoplasmic part of the bacteria is rapidly destroyed, mainly because of osmotic effects[1,2]. Two structural elements are observed in the bacterial suspension after completed lysis: membraneous 'ghosts', probably mainly representing cytoplasmic membranes, and granular material. Relatively few granules are found, however, in cells of the *B. megaterium* strain used in the present work[3].

On the other hand, when cells of *B. megaterium* are treated with lysozyme in 0·2–0·5 *M* sucrose, the osmotic lysis is prevented, and the released bacterial protoplasts remain stable for a considerable time[2]. These protoplasts lyse immediately, however, when transferred to a dilute salt solution.

Under certain conditions lysozyme forms insoluble complexes with substances in the bacterial cell wall[4,5]. This phenomenon, which prevents lysis of the bacteria, has been interpreted in terms of a stable enzyme - subtrate complex[5]. It has also been suggested that lysozyme may form complexes with the cytoplasmic membrane of bacteria, thus giving rise to cytological artefacts[6].

In order to study the affinity of lysozyme to components of *B. megaterium* cells, this enzyme was labelled with iodine-131 according to the procedure described by Day *et al.*[7] It was calculated that, on an average, each molecule of the treated enzyme contained approximately one atom of iodine. As can be seen from Fig. 1, the labelling left the activity of the lysozyme substantially unaffected.

The fractionation of *B. megaterium* cells followed in the main the procedure described by Weibull *et al.*[3] Thus, in a typical experiment 1·6 ml. of 2 *M* sucrose was added to a suspension of 20·2 mgm. (dry weight) of cells in 4·7 ml. phosphate buffer; 0·88 mgm. of labelled lysozyme, dissolved in 0·2 ml. distilled water, was added to this mixture. When the cells had been converted into protoplasts, these bodies were sedimented by centrifugation at 6,000 *g*. The supernatant liquid (cell-wall fraction in Table 1) was withdrawn and the protoplasts were lysed by suspending them in distilled water. The lysate, containing soluble protoplasm and 'ghosts', was centrifuged at 78,000 *g* for 20 min. and the sedimented 'ghosts' were washed three times with distilled water. The supernatant liquid (soluble protoplasm in Table 1) was centrifuged at 103,000 *g*. for 120 min.

The results of the experiment are shown in Table 1. The radioactivities were measured with an Ekco scintillation scaler equipped with a thallium-activated sodium iodide crystal. It can be seen that 91 per cent of the activity of the original lysate was recovered in the various fractions. The main part was found in the cell-wall fraction, but substantial amounts were present also in the soluble protoplasm and in the 'ghost' fraction. When the cell-wall fraction was precipitated with trichloroacetic acid, about 95 per cent of the activity was recovered in the precipitate. Thus the iodine bound to the lysozyme was not released to any appreciable extent during the enzyme reaction. The activity of the fraction containing the soluble protoplasm can be largely explained by the fact that the protoplasts were not washed before being lysed (this could not be done because of their fragility). The activity found in the 'ghost' fraction corresponds to 0·072 mgm. lysozyme. The dry weight of the 'ghosts' was found to be 3·2 mgm. Thus, the lysozyme associated with the 'ghosts' corresponds to 2·3 per cent of the dry weight of these structures. This must imply that the lysozyme present in the 'ghost' fraction can scarcely represent a morphologically or structurally important component of the 'ghosts'.

Table 1. DISTRIBUTION OF LABELLED LYSOZYME BETWEEN FRACTIONS OF *Bacillus megaterium* CELLS.

	Cell material	Activity (per cent of original lysate)
1.	Cell wall fraction	69·4
2.	Soluble protoplasm	12·0
3.	Soluble protoplasm, centrifuged at 103,000 *g*.	10·3
4.	'Ghosts' (washed)	8·2
5.	Wash waters	2·0
6.	Fractions 1+2+4+5	91·6

The activity found in the fraction containing the soluble protoplasm corresponds to 0·11 mgm. lysozyme. Of this amount, 86 per cent remained in the supernatant liquid after centrifugation at 103,000 *g*. for 120 min. (see Table 1). During such a procedure, at least 80 per cent of the so-called '40 *S* particles'[8] which contain ribonucleic acid, are sedimented[3]. Thus, since the 40 *S* particles represent about 10 per cent of the dry weight of the bacteria[8], most of the lysozyme found in the fraction containing the soluble protoplasm would have been sedimented if this enzyme had been associated with the 40 *S* particles.

Thus, no evidence has been obtained during the present work indicating any extensive formation of complex between lysozyme and components of the bacterial cell other than the cell wall.

Several investigations have shown that labelling with iodine leaves the electrophoretic and immunological properties of the treated proteins largely unaffected[9]. However, to our knowledge the present work is the first one dealing with the effects of iodine labelling on the functioning of an enzyme.

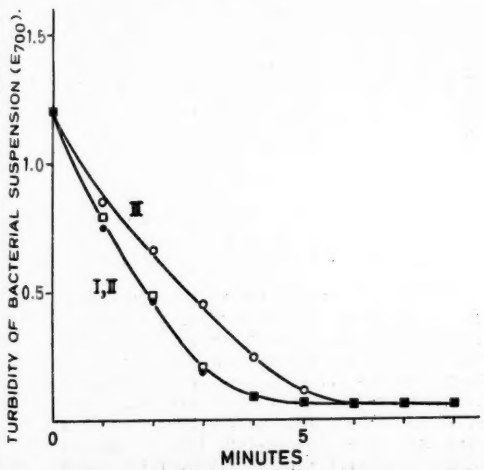

Fig. 1. Lysis of *B. megaterium* cells by lysozyme. ●, 135 μgm. unlabelled lysozyme per ml. ○, 90 μgm. unlabelled lysozyme per ml. □, 135 μgm. lysozyme, labelled with iodine-131, per ml.

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CYTOLOGY

Development of the Yolk Nucleus in the Oocytes of Some Fishes

In the young oocytes of the cyprinids *Barbus everetti* Boulenger and *Barbus fasciatus* Bleeker the ameiurid *Ameiurus nebulosus* Lesueur and the silurid *Synodontis nigriventris* David the development of the so-called yolk nucleus has been examined.

This body originates in the above-mentioned species from the nucleus by means of an extrusion, corresponding to the nucleolar extrusion, which is a well-known phenomenon in the oocytes of fishes[1-5] and amphibians[6].

In the nucleus of the young oocyte (fixed in picroformol-acetic, paraffin sections cut at 4–6 μ thickness and stained iron hæmatoxylin Heidenhain and hæmatoxylin Delafield) an intranuclear body, which is larger than a nucleolus and shows a fair staining intensity, migrates to the periphery, pierces the nuclear membrane, passes through the cytoplasm as the yolk nucleus becomes vacuolated, spongy and loses part of its staining intensity, ultimately to reduce in the cortical layer of the cytoplasm, close to the cell membrane to a granular cytoplasmic inclusion, which is predestined increasingly to lose its staining intensity and ultimately to merge completely with the cytoplasm.

In this intranuclear inclusion two types could be distinguished, namely, the granulated intranuclear body the vacuole of which contains a central granule, and the non-granulated intranuclear body, the vacuole of which lacks this central granule. The development of both types shows some differences. In general, the non-granulated intranuclear body is after extrusion sooner vacuolated than the granulated intranuclear body, whereas also the occurrence of the spongy structure and the decrease in staining intensity occurs sooner.

The first type was observed in *Barbus everetti* and *Barbus fasciatus*, the second in *Ameirus nebulosus* and *Synodontis nigriventris*. As these types correspond very well with those described by Sathyanesan[7] in the cyprinid *Barbus stigma* (Cuv. et Val.) and the silurid *Mystus seenghala* (Sykers), the intranuclear body and the yolk nucleus formed from it possibly have a certain typological significance and this inclusion shows some peculiarities concerning its structure and development in the various fish families.

In the descriptions of some authors[1,2,4] one lacks the migration of this structure towards the periphery of the oocyte, the occurrence of vacuoles and the final complete disappearance.

The nuclear origin of the yolk nucleus had already been suggested by Wheeler[5] and other authors[1].

The functional significance of the extrusion of the intranuclear material according to the general opinion will probably consist just as was observed for the nucleolar extrusion, in a certain metabolism between nucleoplasm and cytoplasm.

How this metabolism is to be imagined, is not such a simple matter, however. In fact, the presence of the ribonucleic acid in the cytoplasm could for example be due in part to a nuclear extrusion, but this would in any event be an exceptional type of mechanism, for Caspersson and Schultz[8] demonstrated that the synthesis of the ribonucleic acid could certainly be carried out direct in the cytoplasm, which is localized next to the nuclear membrane. The possibility exists that this nucleic acid would serve as a quantity of reserve substances, which can be used for the formation of the chromosomes in the segmentation of the mature oocyte.

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MINERALOGY

Salt Desorption from Halloysite

Abnormally high cation exchange capacities (40–70 m.equiv./100 gm.) have been reported for halloysite[1]. Since isomorphous substitution is of inconsiderable magnitude in halloysite, White[2] has suggested that allophane, which sometimes is associated with halloysite, may account for these large values. The recent work of Wada[3], however, shows that salts of potassium and ammonium can replace H_2O from between the layers of hydrated halloysite and form heat-stable complexes with the clay. The amount of salt so held is large (200–300 m.equiv./100 gm.).

I felt that there might be a connexion between this large 'salt-holding capacity' and the high cation exchange capacities variously reported for halloysite. Accordingly, hydrated halloysites from Floyd and from Piney River, Virginia, were saturated with potassium chloride, then washed with 25-ml. portions of either water, 100 per cent methyl alcohol or 95 per cent ethyl alcohol. In addition, similar samples were dehydrated prior to saturation with potassium chloride and washed in the same manner.

Ethyl alcohol was particularly ineffective in removing interlayer salts from the hydrated halloysites. After washing the Floyd sample with 100 ml., 109 m.equiv. of salt/100 gm. remained ; after 200 ml. this had been reduced by only 3 m.equiv. The Piney River halloysite behaved similarly, with values of 81 and 79 m.equiv./100 gm. after 100 and 200 ml. of washing, respectively. Clays washed with methyl alcohol retained about 50 m.equiv. of salt per 100 gm. after washing with 100 ml., but were fairly well cleaned by washing with 250 ml. Water quite effectively removed the bulk of the salt after three washings (75 ml.). Dried halloysite was found to release salt readily when washed either with alcohol or water, implying that the interlayer salt complex discovered by Wada[3] is the site of the salt which is difficult to remove.

It is concluded that the use of ethyl or methyl alcohols as washing reagents, where hydrated halloysite is encountered, is liable to result in incomplete removal of salts. This conceivably could lead to the anomalous cation exchange capacities reported for halloysite in the literature.

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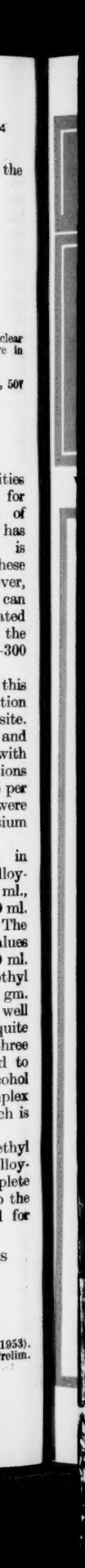
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